JOURNAL OF DAIRY SCIENCE

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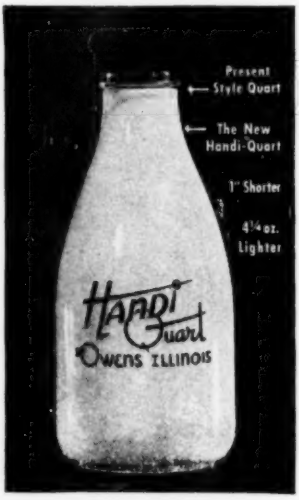

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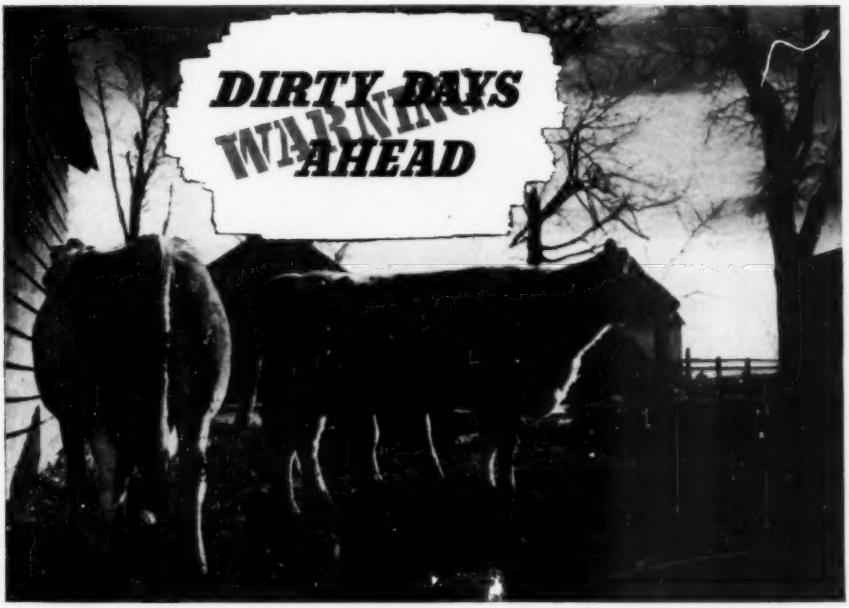

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OFFICIAL ORGAN OF
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Published at
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The Journal of Dairy Science is issued monthly. Subscription is by the volume and one volume is issued per year.

Manuscripts should be typewritten and carefully revised before submission to T. S. Sutton, The Ohio State University, Columbus, Ohio. Fifty reprints will be furnished gratis to authors provided others are ordered. Cost of additional reprints and reprint order blank will be submitted with proof.

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Advertising should be mailed direct to the Science Press Printing Company, N. Queen St. and McGovern Ave., Lancaster, Pennsylvania.

Correspondence regarding business policies of the Journal should be addressed to the Secretary-treasurer, R. B. Stoltz, The Ohio State University, Columbus, Ohio.

Post Office Notices of undeliverable copies and changes of address should be sent to R. B. Stoltz, The Ohio State University, Columbus, Ohio.

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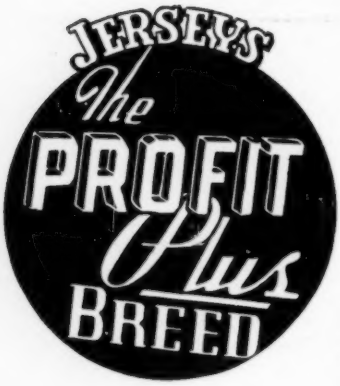

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JOURNAL OF DAIRY SCIENCE

VOLUME XXV DECEMBER, 1942 NUMBER 12

PREDICTING 305-DAY YIELDS FROM SHORT-TIME RECORDS*

C. Y. CANNON, J. B. FRYE, JR., AND JOHN A. SIMS
Division of Dairy Husbandry, Iowa State College, Ames

One of the great difficulties confronting dairymen when purchasing cows is the lack of information about their producing ability. Without production records dependence is placed only on the visible characters of the animals. The relationship of these characters to production and the ability of men to estimate production from type have been examined (2). The discrepancies between the actual yields of milk and fat and the estimates generally have been large.

Since only a small percentage of the cows offered for sale have known records, a quick, easy and accurate method of estimating the producing ability would be useful. It is sometimes possible to get a one-day test from which an estimate could be made if the cow's month to month decline in yield (lactation curve) was known.

Lactation curves, although not all alike because they are affected by certain environmental and inherent factors have so much in common that an average curve can be used to obtain a prediction of yield accurate enough to be useful in supplementing the judgment made from examination of the cow's physical characters. Such information has been used (3, 5) to convert incomplete or partial lactations to 10-month and yearly records.

Our investigation was initiated to obtain factors by which predictions of the 10-months lactation can be made from the yield of any one month or day in the lactation.

EXPERIMENTAL

The data were derived from two sources: The dairy herd maintained by Iowa State College, and the Iowa Dairy Herd Improvement Associations.

College dairy herd material. The first ten months of all normal lactations made in the College Dairy Herd during the period 1908–1938 were used. Every record beginning with an abnormal calving or influenced by serious sickness was discarded. Prediction factors for production were determined by using only 400 records, all made by Holstein-Friesian cows.

Daily milk weights were used in determining monthly milk yields. Dur-

Received for publication June 3, 1942.

* Journal Paper No. J–1019 of the Iowa Experiment Station, Ames, Iowa. Project No. 31.

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ing part of the time, composite weekly samples of milk were used in determining the fat percentages from which the monthly yields of butterfat were computed. During the balance of the time, the fat percentages were determined from the composite sample (sometimes calculated from the fat determinations of each milking) of only one day's milk yield each month. All records were used as actually made without any corrections for age, etc.

Dairy herd improvement association material. The large proportion of the 1289 lactations which came from five breeds (grades and purebreds) were made by Holsteins (554 lactations), Guernseys (422 lactations) and Jerseys (199 lactations). Only the first ten months of each lactation were used. Some of the lactations were shorter than this though no record that was not a complete lactation was used.

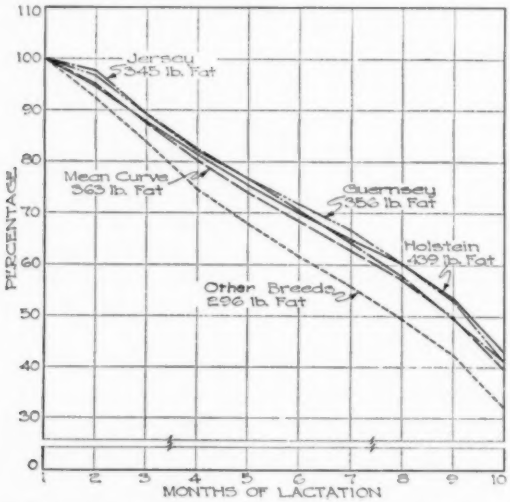

FIG. 1. Curves showing the perentage relationship of each month's yield to that of the first month for cows in the Iowa State College herd.

In Dairy Herd Improvement Association testing, a single day's milk yield and its butterfat percentage is obtained from which the month's yield is calculated. Since each month's yield is a multiple of its day's yield, calculations of the lactation curves on a percentage basis would be the same when based on the day's yield as when based on the month's yield. Factors of prediction determined from the days' yields also would have the same relation to each other as factors determined from the months' yields.

In Iowa, each D.H.I.A. supervisor is required to record originally milk yield and butterfat percentage for each cow in a herd, breeding and calving dates, and unusual conditions affecting the cow's production on a "barn sheet," carbon copies of which are forwarded to the state supervisor's office each month. These supplied the data for this investigation.

As in the case of the college records all Dairy Herd Improvement Association records were used without any correction, though the records were made by cows at all ages.

RESULTS AND DISCUSSION

Although only the Holstein-Friesian records made in the college herd were used for the calculation of equations for predicting yield (regression equations) the records of other breeds in the herd were analyzed to determine if there were marked breed differences in the slope of the lactation curves.

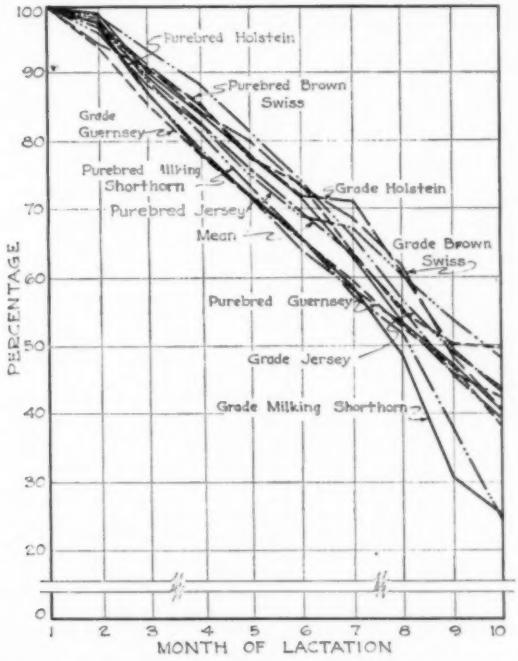

FIG. 2. Curves showing the percentage relationship of each month's yield to that of the first month for cows of different breeds in the D.H.I.A.

Figure 1, shows a great similarity between the curves of the Guernseys, Holsteins and Jerseys. The other breeds failed to show the same persistency but the records involved were so few their curves may not be typical.

The same similarity in the lactation curves of the different breeds was shown by the records made in the Dairy Herd Improvement Association (fig. 2). Because of this no segregation of records by breeds was made in the calculation of prediction factors (regression equation) from the Dairy Herd Improvement Association data.

The essential items obtained in the statistical analyses of both sets of data are presented in tables 1 and 2.

TABLE 1

Data on 400 Holstein-Friesian lactations showing the linear regression coefficient, regression equation, standard error of estimate and correlation

(Iowa State College herd data)

Month of lactation	No. of records	$\bar{x}$	σx	$\bar{y}$	σy	Regression equation*	Standard error of estimate	Correlation coefficient
1–A	400	50.4	12.5	369.1	108	4.99A + 117	73.2	0.74
2–B	400	47.8	14.2	369.1	108	6.02B + 81	66.6	0.79
3–C	400	44.3	12.9	369.1	108	7.19C + 51	56.1	0.86
4–D	400	41.2	12.6	369.1	108	7.53D + 59	51.6	0.88
5–E	398	38.3	11.7	370.1	107	8.40E + 49	43.8	0.91
6–F	397	35.6	11.5	370.5	107	8.50F + 68	44.4	0.91
7–G	390	33.7	11.2	372.4	107	8.55G + 84	46.1	0.90
8–H	388	31.4	11.2	373.1	107	8.28H + 113	52.1	0.87
9–I	374	28.8	11.8	377.1	105	7.01I + 175	65.2	0.79
10–J	350	24.8	12.7	381.1	106	5.61J + 242	78.9	0.67

* When A equals fat yield of first month, B equals fat yield of second month, etc.

TABLE 2

Data on 1289 lactations showing the linear regression coefficient, regression equation, standard error of estimate and correlation (D.H.I.A. records)

Month of lactation	No. of records	$\bar{x}$	σx	$\bar{y}$	σy	Regression equations*	Standard error of estimate	Correlation coefficient
1–A	1289	1.64	0.47	11.17	2.82	4.08A + 135	61.5	0.40
2–B	1289	1.52	0.43	11.17	2.82	5.13B + 101	51.9	0.50
3–C	1289	1.40	0.39	11.17	2.82	6.00C + 83	47.7	0.59
4–D	1289	1.30	0.36	11.17	2.82	6.41D + 85	48.0	0.63
5–E	1289	1.21	0.34	11.17	2.82	5.06E + 152	45.6	0.69
6–F	1289	1.12	0.32	11.17	2.82	5.55F + 149	46.8	0.72
7–G	1287	1.02	0.33	11.18	2.80	6.55G + 135	48.0	0.70
8–H	1245	0.91	0.33	11.29	2.78	7.18H + 143	56.7	0.64
9–I	1093	0.79	0.35	11.56	2.89	7.25I + 175	73.5	0.48
10–J	791	0.70	0.30	11.92	2.79	6.43J + 223	74.7	0.68

* When A equals fat yield of the test day in first month, B equals fat yield of the test day in second month, etc.

Table 3 shows the decline in yield from month to month, of the cows in the college herd and in the Dairy Herd Improvement Associations. These figures calculated as percentages of the first month's yield and as percentages of the previous month's yield indicate some differences in persistency between the two groups. The persistency index of the College cows was 92.3 and for the D.H.I.A. cows 91.0 when calculated according to Turner's method (4).

TABLE 3

Month to month decline in yield of College and D.H.I.A. cows calculated as percentage of the first month yield and as percentage of the previous month's yield

Month of test	College data			D.H.I.A. data		
	Yield	Per cent of 1st month	Per cent of previous month	Yield	Per cent of 1st month	Per cent of previous month
1	50.4	100.0	.....	1.64	100.0	.....
2	47.8	94.8	94.8	1.52	92.7	92.7
3	44.3	87.8	92.7	1.40	85.4	92.1
4	41.2	81.7	93.0	1.30	79.3	92.9
5	38.2	75.8	92.7	1.21	73.8	93.1
6	35.6	70.6	93.2	1.12	68.3	92.6
7	33.7	66.8	94.7	1.02	62.2	91.1
8	31.4	62.3	93.2	0.91	55.5	89.2
9	28.8	57.1	91.7	0.79	48.2	86.8
10	24.8	49.2	86.1	0.70	42.7	88.6
	Persistency index 92.3			Persistency index 91.0		

The prediction of yield according to our results is most accurately made from a test taken during the fifth month of lactation as shown by the low standard error of estimate. This is true both with records obtained from the College herd and from D.H.I.A. herds (tables 1 and 2). The tests made in the sixth month gave the next most accurate predictions, followed in turn by those made in the seventh and fourth months. These results varied somewhat from those obtained by Gaines (1) and Kartha (3) who found that a short-time test taken in the fourth month after calving gave the most accurate basis for estimating a 305-day record. There is less accuracy in predicting yield when based on tests made in the first or last months of the 10-months lactation than there is when based on tests made nearer the middle of the lactation.

Sometimes one wishes to estimate what a cow might produce when only a part of the lactation record is known. Occasionally a cow will die or become seriously injured during a lactation and her 10-month record is wanted in the proving of her sire. Factors for these estimates were made from our data. They are found in table 4 and are for estimating the ten months yield of butterfat.

Dairy men may use the regression equations in tables 1 and 2 as factors for predicting 10-month yields of dairy cows when only one day's yield is

known. The day's yield of butterfat is multiplied by 30.5, the average number of days in a month, to obtain the month's yield. The elapsed time between 22 days after the day of calving[1] and the day on which the yield is taken will determine the month of lactation which will also determine the factor to use in making the estimation of yield.

In table 5 are records of cows selected at random from four herds in Iowa Dairy Herd Improvement Associations. Variations occur between the estimated yields (columns 9 and 10) and the actual yields (column 11) which are sometimes rather large (cow 12) while other estimates are very close to the actual yield (cow 3). It is not expected that the estimated yield for every single cow will be as close to the actual as is the case with cow 3.

TABLE 4

Factors for correcting partial lactations to ten months lactations (butterfat yield basis)*

Months in milk	Factors based on data collected from	
	College herd	D.H.I.A. herds
1	7.47	7.08
2	3.83	3.67
3	2.64	2.55
4	2.05	1.98
5	1.70	1.64
6	1.46	1.42
7	1.29	1.26
8	1.17	1.15
9	1.07	1.06

* Multiply the total yield for the number of months in milk by the corresponding factor.

If a few cows are used then the variations in one direction tend to cancel out those in the other. In the case of the fifteen cows used in table 5 the total estimated yield for all the cows is 132 pounds or an average per cow of 8.8 pounds of fat greater than the actual yield when the "College" factors are used and 46 pounds or an average of 3.0 pounds of fat less when the "D.H.I.A." factors are used.

It would be an unusual person who could estimate the probable yield of these fifteen cows as closely as this from a mere physical examination of them. The little extra effort of taking a single day's milk yield and butterfat test on each cow permits knowing rather accurately what to expect in the way of production from the lactations the cows are passing through. The physical examination and the test with the accompanying estimated yields give a much greater basis for judgment than either alone.

Because the regression coefficients and the factors for correcting partial lactations calculated from the College herd data (tables 1 and 4) were from

[1] There is a lag of 22 days from calving until the first testing period in D.H.I.A. records.

TABLE 5

Estimated yields (fat) of unselected cows calculated from factors derived from College and D.H.I.A. data compared with actual yields taken from the D.H.I.A. records

1	2	3	4	5	6	7	8	9	10	11
Cow No.	Breed	Date of freshening M–D–Y	Date of test M–D–Y	Months in lactation*	Day's milk (lbs.)	Production fat		Estimated 305-day fat yields, in lbs, using		Actual 305-day fat yields in lbs. according D.H.I.A.
						Per cent	Lbs.	College factors	D.H.I.A. factors	
1	G.H.	8– 3–40	4–21–41	8	27.8	3.1	0.86	330	331	310
2	G.H.	3– 3–41	4–21–41	1	66.3	3.0	1.99	420	383	424
3	G.H.	2– 1–41	4–21–41	2	38.2	3.0	1.14	290	279	283
4	R.H.	11–30–40	4–21–41	4	39.4	3.1	1.22	339	323	310
5	G.H.	1– 2–41	4–21–41	3	50.1	3.5	1.75	434	403	437
6	G.J.	1–19–41	3– 2–41	1	33.1	6.3	2.08	433	394	452
7	G.G.	10–25–40	8–18–41	10	16.6	6.5	1.07	425	433	493
8	G.G.	11–20–40	8–18–41	9	19.8	4.8	0.95	378	385	443
9	G.G.	10–29–40	8–18–41	9	17.2	4.3	0.73	331	336	297
10	G.G.	11– 1–40	8–18–41	9	16.8	5.5	0.92	372	379	346
11	G.H.	9–22–40	5– 6–41	7	31.9	2.9	0.93	327	321	356
12	R.H.	10–28–40	5– 6–41	6	41.5	3.8	1.58	478	417	372
13	G.H.	12–27–40	5– 6–41	4	37.2	2.9	1.08	307	280	219
14	R.H.	9–17–40	5– 6–41	7	24.8	2.7	0.67	258	269	293
15	.....	10–12–40	5– 6–41	6	24.5	3.2	0.78	270	281	225
						Total		5392	5214	5260
						Total error		+ 132	– 46	
						Aver. error		+ 8.8 lbs. per cow	– 3.0 lbs. per cow	

* An easy way to calculate the "months in lactation" is to subtract the date of freshening from the date of test as

Year	Month	Day	
40 41	16 4	21	This indicates that 8 months and 18 days have elapsed
40	8	3	
—	—	—	
	8	18	

between the date of calving and the date of test. Since there is a lag of 22 days the factor to be used should be that for the 8th month. Had the number of days beyond 8 months exceeded 22 days then the factor for the 9th month would have been used.

records made by Holstein cows only, their use should not be as generally applied as those calculated from the Dairy Herd Improvement Association data (tables 2 and 4). These latter were calculated from records made under more general conditions so their use would give more conservative results and would better fit the conditions of most farmers' herds.

In reality the variations in records calculated from the two sets of factors are not great and either can be used successfully.

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GRAPHIC METHOD SHOWING EFFICIENCY OF DAIRY BULLS

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Dairy bulls are commonly evaluated by comparing the yields of daughters and dams. The result is usually expressed as an index which is supposed to represent an estimate of what the production of the bull's daughters would be if they were not influenced by the hereditary effect of their dams.

Such an index is concise and valuable but for educational purposes in classes or farmers' meetings it may be helpful to illustrate the efficiency of bulls by a graphic method such as that used in the accompanying chart.

In this chart the butterfat production of dams and daughters from six different bulls is shown. The records are from a small dairy herd over a period of years.

The graphs are so designed that when the records of a dam and daughter are equal, the dot will fall on the diagonal line; if the daughter's production

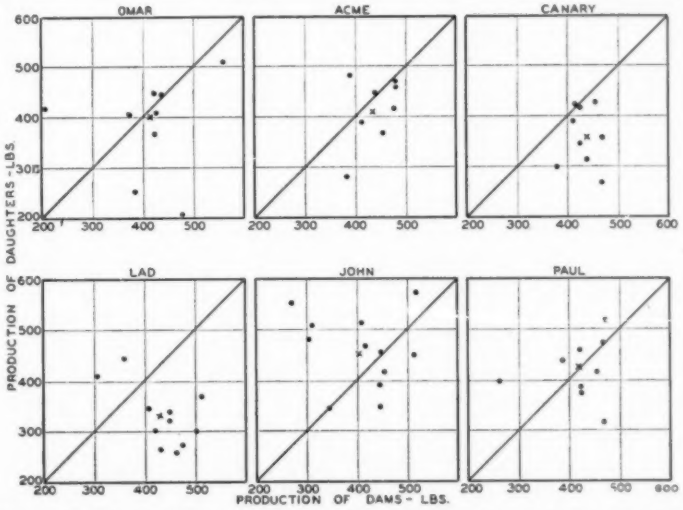

FIG. 1. Effect of bulls on production in a dairy herd. Each dot represents the production of a dam and her daughter. X represents the average of dams and daughters.

is greater than the dam's the dot will be above, while if it is less than the dam's it will be beneath the diagonal line. By noticing the position of the dots one can tell at a glance whether the bull is increasing or decreasing the production of the herd.

Received for publication June 4, 1942.

Note for instance, that in the Canary and Lad diagrams the dots are almost entirely beneath the diagonal line and indicate the extent to which the daughters were inferior to their dams. On the other hand, John was a superior bull with eight out of twelve daughters equal to or superior to their dams.

The average production of any group of dams and daughters is indicated by the position of the X within each major square representing any given bull.

The position of the X indicates that of the six dairy bulls, only John and Paul increased the average productivity of the herd. Canary and Lad were inferior bulls that reduced the average production of the herd to a remarkable degree. It is interesting to note that Canary was a show bull that had won some ribbons at fairs but his value lay chiefly in his looks, not in his performance.

The reliability of the average production of the daughters from each bull can be estimated by the compactness of grouping of the dots.

THE ADDITION OF CAROTENOIDS AS A MEANS OF MAINTAINING A UNIFORM COLOR IN MILK AS MEASURED BY THE LAC–CHROM–METER

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For a long time milk consumers have noticed that the milk which they buy varies in color throughout the year. Milk normally has a deeper yellow color during the spring and summer when cows have access to fresh green pasture.

In this investigation color determinations of milk of the five breeds, Ayrshire, Guernsey, Holstein, Jersey and Brown Swiss were made using a Munsell "Lac-chrom-meter."[2] Figure 1 shows a diagrammatical sketch of the instrument used. The procedure for making the color determination is briefly as follows: the instrument is properly balanced, the sample cell C is filled with the milk to be tested, placed on the sample shelf SH, and the light B moved until the spot S in the center of the field disappears. The point where the pointer shadow falls on the scale indicates the color grade of the sample measured. Garrett *et al.* (3) have given the principles involved in the construction and operation of a similar instrument known as a "lactochrometer."

The effect of the direct addition of carotene on the color of the milk was also measured with the same instrument in an effort to determine the feasibility of standardizing the color of milk throughout the year by the direct addition of carotene during seasons when the normal color of the milk is low. At various intervals carotene determinations on the normal milk were made in an effort to find out if there was any correlation between the color of the milk as determined on the "Lac-chrom-meter" and the carotene content of the same milk.

Baumann and Steenbock (2) found that "carotene addition to winter butter in an attempt to increase the vitamin A activity up to that of summer butter does not appear to be practical unless the public should be found to be willing to accept a much more highly colored product than at present."

The milk from the various breeds was collected from four or more cows of the same breed in the Ohio State University dairy herd. The cows were stabled from the middle of November until April 21 at which time they were

Received for publication June 6, 1942.

[1] Now located at Colorado State College, Fort Collins, Colorado.

[2] The instrument used was loaned through the courtesy of the American Guernsey Cattle Club.

turned out on pasture. During the period from February 17 to April 21, they received 24 to 36 pounds of molasses alfalfa silage, 7 to 12 pounds of grain and all the good quality leafy green alfalfa hay they would eat. The mixed herd samples, which were tested, were taken from the University herd mixed-milk after it had been delivered to the University dairy and dumped into the weigh tank. By using such milk, a representative mixed milk could be obtained as the University herd is made up of cows from the five breeds named above. The homogenized milk used was the same mixed milk after it had been homogenized and pasteurized.

When readings were made on the various milks standardized to five per cent fat, a portion of the milk was separated by gravity and the cream and skimmilk recombined in the amounts necessary to produce a milk testing five per cent.

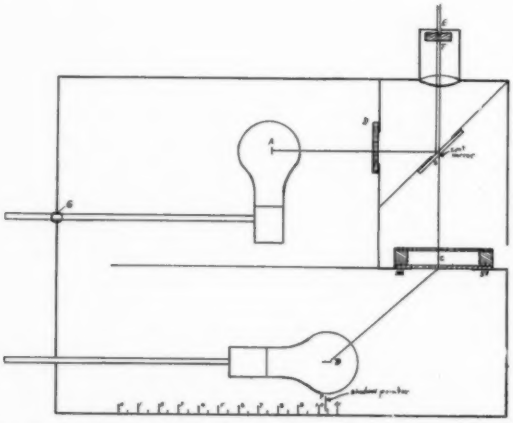

FIG. 1. Diagrammatical sketch of the "Lac-chrom-meter."

The source of carotene used was "Provalac," a commercial beta carotene concentrate emulsified in butterfat, furnished by the General Biochemicals Company, of Chagrin Falls, Ohio. It was diluted by mixing one part of the concentrate with nine parts of homogenized milk. This solution was then pipetted into the samples at the rate necessary to give an addition of 300, 600 and 900 micrograms carotene per quart, equal to 500, 1000 and 1500 International Units of vitamin A, respectively.

For the carotene determinations, a portion of the natural milk was separated by gravity, the cream being churned into butter which was analyzed for carotene content spectrophotometrically at the Ohio Agricultural Experiment Station.

The results of these experiments are given in table 1. According to the "Lac-chrom-meter" readings, Guernsey milk was consistently higher in

color than that of the other breeds, followed in order by Jersey, Ayrshire and Brown Swiss (the latter two being approximately the same), and Holstein which showed the least color.

From February 17 until April 21, at which time the cows were turned on pasture, the color of the milk within the various breeds varied to some extent but was generally lower than the color exhibited by the same breed after the cows were turned on pasture. This increase in color is attributed to the increased carotene content of the milk due to the cows being placed on green pasture. The milk of the Jersey and Guernsey breeds increased in color to a greater extent than that of the other breeds. The results indicate that the milk of each breed reached its peak in color approximately three weeks after the cows were turned on pasture, and after the peak was reached, there was a slow but general decline in color into August for some of the breeds, yet the carotene content of the milk actually increased as shown in table 1.

The results presented in table 1 show that by adding carotene to the milk of the various breeds or to mixed herd milk, the color of the milk is increased by approximately 1.0 "Lac-chrom-meter" unit with each addition of 300 micrograms of carotene. On this basis it would have required the addition of about 450 micrograms of carotene per quart to the winter milk to bring about a color somewhere near the maximum obtained when the cows were on pasture.

Since the color of milk may be increased by adding carotene directly to the milk, visual comparisons of the various types of milk used in this experiment were made using normal Guernsey milk as a standard. Samples of the various types of milk were placed in test tubes of the same diameter and carotene was added to them until they matched the color of the normal Guernsey milk according to a visual comparison. Carotene in the following amounts per quart of five per cent milk was needed to match the Guernsey milk in most instances: 600 micrograms for Ayrshire, Holstein, and Brown Swiss; 180 for Jersey; 450 for mixed herd milk; and 300 for the same mixed herd milk after it was homogenized. The color of the milk to which carotene was added did not exactly match the color of the Guernsey milk, as it had somewhat of a reddish tinge, slightly different from the normal yellow color of the Guernsey milk. The visual color seemed to increase to a greater extent on the addition of carotene than did the "Lac-chrom-meter" units.

The comparison between the "Lac-chrom-meter" reading and carotene content shows only a general correlation in this respect and not a direct one, as an inspection of table 1 will show. This indicates that the "Lac-chrom-meter" reading is influenced by other factors than the carotene content of the milk. That there is a correlation between the fat content and color of milk has been shown by Bartlett *et al.* (1). The fat content of the milk affected the "Lac-chrom-meter" reading. This point is illustrated in table

TABLE 1

Color determinations as made on the Munsell "Lac-chrom-meter."
Results expressed as Lac-chrom-meter units

Raw Ayrshire Milk

Date	Normal milk			Normal milk with carotene added (Lac-chrom-meter units)			Standardized to 5% fat	Micrograms of carotene per quart to bring visual color of milk to that of Guernsey (both milks standardized to 5% fat)
	Fat content	Lac-chrom-meter units	Carotene content (mg./kg. fat)	300 μg.	600 μg.	900 μg.	(Lac-chrom-meter units)	
2–17–41	.....	1.0	.....	2.2	3.3	4.4	.....	.....
2–25–41	4.1	1.1	1.04	2.2	3.3	4.3	.....	.....
3–13–41	4.1	1.8	.....	2.9	4.0	5.0	.....	.....
3–29–41	4.3	1.1	.....	2.1	3.1	4.1	.....	.....
4–14–41	3.8	1.1	.....	2.1	3.1	4.1	.....	.....
4–20–41	3.7	1.0	1.50	2.0	4.1	4.1	.....	.....
5– 7–41	3.9	2.0	3.08	3.0	4.0	5.0	.....	.....
5–14–41	4.3	2.4	.....	3.4	4.4	5.4	2.9	600
5–27–41	4.0	1.9	.....	2.8	3.9	4.9	3.0	600
6– 2–41	3.9	1.8	.....	2.7	3.7	4.8	3.5	600
6–17–41	4.0	1.5	.....	2.4	3.4	4.4	2.9	600
7–28–41	4.0	1.4	3.65	2.3	3.4	4.4	2.8	600

TABLE 1.—(*Continued*)

Raw Brown Swiss Milk

Date	Normal milk			Normal milk with carotene added (Lac-chrom-meter units)			Standardized to 5% fat	Micrograms of carotene per quart to bring visual color of milk to that of Guernsey (both milks standardized to 5% fat)
	Fat content	Lac-chrom-meter units	Carotene content (mg./kg. fat)	300 μg.	600 μg.	900 μg.	(Lac-chrom-meter units)	
2–17–41	.....	0.9	.....	2.0	3.0	4.1	.....	.....
2–25–41	4.0	0.6	1.58	1.6	2.5	3.6	.....	.....
3–13–41	4.3	1.9	.....	3.0	4.0	5.0	.....	.....
3–29–41	4.2	1.1	.....	2.0	3.2	4.2	.....	.....
4–14–41	3.5	0.6	.....	1.6	2.6	3.6	.....	.....
4–20–41	3.5	0.5	1.92	1.6	2.6	3.5	.....	.....
5– 7–41	3.7	1.6	4.40	2.5	3.4	4.3	.....	.....
5–14–41	4.5	2.5	.....	3.6	4.5	5.5	2.8	600
5–27–41	4.0	1.8	.....	2.7	3.8	4.8	3.0	600
6– 2–41	4.0	1.8	.....	2.7	3.7	4.6	3.6	600
6–17–41	3.7	1.5	.....	2.5	3.4	4.4	3.3	600
7–28–41	3.8	1.4	4.20	2.4	3.4	4.4	3.2	600

TABLE 1.—(*Continued*)

Raw Guernsey Milk

Date	Normal milk			Normal milk with carotene added (Lac-chrom-meter units)			Standardized to 5% fat	Micrograms of carotene per quart to bring visual color of milk to that of Guernsey (both milks standardized to 5% fat)
	Fat content	Lac-chrom-meter units	Carotene content (mg./kg. fat)	300 μg.	600 μg.	900 μg.	(Lac-chrom-meter units)	
2–17–41	.....	3.1	.....	4.0	5.0	5.9	.....	
2–25–41	4.9	2.6	3.38	3.6	4.6	5.6	.....	
3–13–41	4.7	3.8	.....	4.8	5.7	6.6	.....	
3–29–41	4.9	3.1	.....	4.1	5.0	5.9	.....	
4–14–41	4.6	2.1	.....	3.1	4.2	5.1	.....	
4–20–41	4.6	2.1	3.76	3.1	4.2	5.2	.....	
5– 7–41	4.2	5.1	9.85	6.0	6.8	7.4	.....	
5–14–41	4.9	6.0	.....	7.0	7.5	8.0	6.1	
5–27–41	4.5	5.5	.....	6.4	7.4	8.4	6.0	
6– 2–41	4.6	5.7	.....	6.7	7.6	8.5	6.5	
6–17–41	5.2	6.0	.....	7.0	7.9	8.9	6.0	
7–28–41	5.0	5.8	12.15	6.7	7.7	8.7	5.8	

TABLE 1.—(*Continued*)

Raw Holstein Milk

Date	Normal milk			Normal milk with carotene added (Lac-chrom-meter units)			Standardized to 5% fat	Micrograms of carotene per quart to bring visual color of milk to that of Guernsey (both milks standardized to 5% fat)
	Fat content	Lac-chrom-meter units	Carotene content (mg./kg. fat)	300 μg.	600 μg.	900 μg.	(Lac-chrom-meter units)	
2–17–41	.....	0.6	.....	1.5	2.6	3.7	.....	.....
2–25–41	4.7	0.0	1.28	0.7	1.6	2.5	.....	.....
3–13–41	3.4	0.3	.....	1.2	2.1	3.1	.....	.....
3–29–41	3.3	0.0	.....	0.6	1.5	2.4	.....	.....
4–14–41	3.5	0.0	.....	0.5	1.4	2.3	.....	.....
4–20–41	3.4	0.0	1.18	0.6	1.5	2.4	.....	.....
5– 7–41	3.2	0.0	2.98	0.9	1.8	2.7	.....	.....
5–14–41	3.5	0.7	.....	1.6	2.6	3.6	2.0	600
5–27–41	3.3	0.5	.....	1.4	2.3	3.3	1.9	600
6– 2–41	3.2	0.6	.....	1.5	2.4	3.4	1.5	600
6–17–41	3.5	0.5	.....	1.4	2.4	3.4	1.9	600
7–28–41	3.5	0.5	3.55	1.4	2.4	3.4	1.8	600

TABLE 1.—(*Continued*)

Raw Jersey Milk

Date	Normal milk			Normal milk with carotene added (Lac-chrom-meter units)			Standardized to 5% fat	Micrograms of carotene per quart to bring visual color of milk to that of Guernsey (both milks standardized to 5% fat)
	Fat content	Lac-chrom-meter units	Carotene content (mg./kg. fat)	300 μg.	600 μg.	900 μg.	(Lac-chrom-meter units)	
2–17–41	.....	3.0	.....	4.0	4.9	5.8	.....	.....
2–25–41	6.2	3.0	3.62	4.0	5.0	6.0	.....	.....
3–13–41	4.3	2.0	.....	3.0	4.0	5.1	.....	.....
3–29–41	5.0	2.1	.....	3.1	4.0	5.0	.....	.....
4–14–41	5.0	1.6	.....	2.6	3.6	4.5	.....	.....
4–20–41	5.0	1.6	3.12	2.6	3.6	4.5	.....	.....
5– 7–41	4.2	3.3	7.02	4.2	5.0	5.8	.....	.....
5–14–41	5.8	5.5	.....	6.4	7.4	8.2	5.0	180
5–27–41	5.7	5.5	.....	6.4	7.4	8.4	4.9	.....
6– 2–41	5.7	5.2	.....	6.1	7.1	7.0	4.8	180
6–17–41	5.5	5.0	.....	6.1	7.0	8.0	4.8	180
7–28–41	5.5	4.8	8.65	5.9	6.9	7.8	4.6	180

TABLE 1.—(*Continued*)

Raw Mixed Herd Milk

Date	Normal milk			Normal milk with carotene added (Lac-chrom-meter units)			Standardized to 5% fat	Micrograms of carotene per quart to bring visual color of milk to that of Guernsey (both milks standardized to 5% fat)
	Fat content	Lac-chrom-meter units	Carotene content (mg./kg. fat)	300 μg.	600 μg.	900 μg.	(Lac-chrom-meter units)	
2–25–41	4.2	1.6	.....	2.5	3.6	4.6	.....	.....
5– 7–41	4.1	3.8	7.10	4.7	5.6	6.5	.....	.....
5–14–41	4.6	3.4	.....	4.5	5.4	6.4	3.6	600
5–27–41	4.2	3.2	.....	4.2	5.3	6.4	4.0	450
6– 2–41	4.3	3.3	.....	4.4	5.4	6.4	4.5	450
6–17–41	4.3	3.2	.....	4.3	5.3	6.3	4.4	450
7–28–41	4.1	3.0	6.55	4.0	5.1	6.1	4.1	450

TABLE 1.—(*Continued*)

Pasteurized—Homogenized (2500 lbs.) Mixed Herd Milk

Date	Normal milk		Normal milk with carotene added (Lac-chrom-meter units)			Micrograms of carotene per quart to bring visual color of 4% milk to that of 5% Guernsey
	Fat content	Lac-chrom-meter units	300 μg.	600 μg.	900 μg.	
2–17–41	4.0	4.3	5.2	6.3	7.3	.....
2–25–41	4.0	4.4	5.3	6.3	7.3	.....
3–13–41	3.9	4.3	5.3	6.4	7.4	.....
3–29–41	4.0	4.3	5.4	6.5	7.6	.....
4–14–41	4.0	4.2	5.2	6.3	7.2	.....
4–20–41	4.0	4.3	5.2	6.2	7.2	.....
5– 7–41	4.0	5.7	6.8	7.8	8.8	.....
5–14–41	5.0	5.8	6.7	7.7	8.6	300
5–27–41	3.9	5.4	6.5	7.5	8.5	450
6– 2–41	3.9	5.6	6.5	7.5	8.5	300
6–17–41	4.0	5.8	6.9	8.0	9.0	300
7–28–41	4.0	5.6	6.6	7.6	8.6	300

1 and shows that the "Lac-chrom-meter" reading of the normal milk is different from that obtained when the milk was standardized to five per cent fat. No attempt was made to standardize the solids-not-fat.

Another important factor affecting the "Lac-chrom-meter" readings is the size of the fat globules in the milk, which is illustrated in table 1 where the readings obtained on the unhomogenized mixed herd milk are compared with the same milk after homogenization at 2500 pounds pressure. Homogenization at the above pressure causes an increase of more than 2.0 "Lac-chrom-meter" units over the corresponding unhomogenized milk. Although the homogenized milk has a richer color than the same milk unhomogenized, it is not in proportion to the increase in "Lac-chrom-meter" units.

Table 2 shows the increase in "Lac-chrom-meter" readings as the

TABLE 2

Lac-chrom-meter reading and size of fat globules as influenced by various homogenization pressures. Mixed herd milk

Homogenization pressure	Lac-chrom-meter reading	Range of fat globule size (microns)	Size of majority of fat globules (microns)
Control—no pressure	3.1	0.5 – 10	5.0
500 lbs.	4.4	0.5 – 6	4.0
1000 lbs.	5.4	0.5 – 5	3.0
1500 lbs.	5.9	0.5 – 3	1.5
2000 lbs.	6.4	0.5 – 3	1.0
2500 lbs.	6.4	0.5 – 3	1.0
3000 lbs.	6.4	0.5 – 3	1.0
3500 lbs.	6.8	0.5 – 2	Less than 1.0
4000 lbs.	6.8	0.5 – 2	Less than 1.0

homogenization pressure is increased and the average size of the fat globules is decreased. These results indicate that as the fat globules are made smaller with a corresponding increase in numbers, the "Lac-chrom-meter" reading increases.

SUMMARY AND CONCLUSIONS

Guernsey milk showed the highest amount of color according to the "Lac-chrom-meter" reading and a visual inspection. Green pasture increased the color of the milk of all the breeds studied. "Provalac" (a carotene concentrate) added directly to the milk increased the "Lac-chrom-meter" reading approximately 1.0 unit for each 300 micrograms of carotene added per quart of milk. It would require about 450 micrograms of carotene per quart to be added to winter milk in order to increase the color to somewhere near the maximum obtained when cows are on pasture. The "Lac-chrom-meter" reading cannot be used as a direct measure of the amount of carotene in the milk. Homogenization increased the "Lac-chrom-meter" reading. The higher the pressure, the greater the reading. The visual color was not increased in proportion to the "Lac-chrom-meter" reading. In the case of

homogenization the "Lac-chrom-meter" reading increased proportionally more than did the visual color, while when carotene was added the visual color increased in greater proportion than did the "Lac-chrom-meter" reading.

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THE EFFECT OF AMPHYL ON MOTILITY AND LONGEVITY OF BOVINE SPERMATOZOA*

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A common recommendation in the practice of artificial insemination of dairy cows is that all contact of semen with chemicals should be avoided. This precaution has applied especially to acids, alkalies, disinfectants, germicides, and such chemical compounds as may give off fumes that are corrosive in nature. A general recommendation of this kind undoubtedly is sound, but it is not based on complete experimental evidence of the effect of chemicals on spermatozoa.

In a series of experiments conducted to determine the value of certain disinfectants in maintaining dairy farm hygiene, an attempt was made to substitute Amphyl for heat in the sterilization of equipment and glassware used in the practice of artificial insemination. Amphyl (1, 2) is the registered trade name of a disinfectant consisting of a mixture of p-chloro-symm.-m-dimethyl hydroxybenzene, p-tert.-amyl hydroxybenzene, and neutral soap in a solution containing 20 per cent (by volume) of alcohol, 6.5 per cent of glycerol, and 34 per cent of water. The concentrated solution has a phenol coefficient of 10. Evidence of its non-toxic, non-injurious action to human and animal tissue and its efficacy as a disinfectant has been published (3, 4).

EXPERIMENTAL PROCEDURE

The semen from one Holstein bull was used in the experiments reported here. In all cases the sperm were 95 per cent motile immediately following ejaculation. In determining the percentage of motility, the semen was transferred to a glass microscope slide by means of a sterile standard wire loop. The glass slide was warmed to about body temperature and the semen was covered with a thin glass slip before examination was made under the microscope. Both the high-dry and oil-immersion objectives of the microscope were used.

The semen, unless otherwise stated, was diluted with a standard diluter solution consisting of equal parts, by volume, of egg yolk and phosphate buffer. The phosphate buffer solution was made by dissolving 0.1 gram of anhydrous KH_2PO_4 and 0.39 gram of anhydrous Na_2HPO_4 in 50 ml. of distilled water. The buffer solution was sterilized in the autoclave.

All semen samples were held at 3° C. while in storage.

Received for publication June 8, 1942.

* Journal series paper of the New Jersey Agricultural Experiment Station, Rutgers University, Department of Dairy Husbandry.

The concentrated Amphyl was diluted with water to the desired concentration.

The measures of the effect of the various treatments on sperm were the percentage of motility at any given time and the length of time required for cessation of all motility.

EXPERIMENTAL RESULTS

Seven experimental trials served to demonstrate that the residues left on artificial insemination equipment and glassware after sterilization with Amphyl had no harmful effect on the motility or longevity of the sperm of semen subsequently brought into contact with this equipment. A severer test of the effect of the Amphyl on semen, therefore, seemed indicated.

The addition of one ml. of a one per cent solution of Amphyl to one ml. of undiluted semen caused all sperm motility to cease within 30 seconds. When one ml. of semen was diluted with one ml. of the phosphate buffer solution which contained one per cent of Amphyl, all motility ceased within one hour. When a small volume of the concentrated Amphyl, however, was added to semen that had been diluted with standard diluter, motility persisted for several days. This unexpected behavior led to the organization of five experimental trials in order to observe, more carefully, the action of the disinfectant on semen.

In the first trial, a sample of semen was diluted 1 : 3 with the standard diluter and was divided into 2 parts, one part to be the control sample and one part the experimental sample. One volume of one per cent Amphyl was added to 5 volumes of the experimental sample, which gave a final concentration of 0.167 per cent of the disinfectant in the diluted semen. Bacterial counts in the treated and untreated semen were made on the first, third, and seventh days of storage. The percentages of motile sperm in both samples were determined daily with the exception of the second and fourth days. The results are presented in table 1.

The rate of decrease in percentage of sperm motility was identical for both the control and the Amphyl-treated semen during the first 5 days of storage. Thereafter, however, the rate of decrease was considerably less in the treated semen than in the untreated semen. All motility had ceased in the control sample by the seventh day, whereas, a small percentage of sperm were still motile in the treated semen, on the tenth day. The bacteriological data indicate that the disinfectant had little influence on the bacteria under the conditions prevailing in this experiment.

In trial 2, a 1 : 1 dilution of the semen was made with the standard diluter. The diluted semen was divided into 3 equal portions of 4 ml. each and put into glass tubes. Tube 1 served as the control. One ml. of one per cent Amphyl was added to tube 2; 2 ml. were added to tube 3. The concentration of Amphyl in tubes 2 and 3 was, respectively, 0.2 and 0.33 per cent.

The results of the motility and bacteriological examinations and of the pH determinations are shown in table 2.

The rate of decrease in motility was about the same for the control and Amphyl-treated samples of semen for the first 4 days. Thereafter the Amphyl-treated semen showed a slower decrease in motility than the control semen. All motility had ceased in the control semen after the seventh day, in the semen containing 0.2 per cent Amphyl after the eleventh day, and in the semen containing 0.33 per cent Amphyl after the fourteenth day. It is interesting to note the length of time motility persisted in a small number of sperm in the semen containing 0.33 per cent of Amphyl. The difference in the numbers of bacteria in the three samples did not seem significant.

TABLE 1

Effect of Amphyl on the percentage of motile sperm, the persistence of sperm motility, and the numbers of bacteria in semen—trial 1

Days	Motility		Bacteria per ml. of undiluted semen	
	Control	Treated	Control	Treated
	%	%	*thousands*	*thousands*
1	80	80	1,840	960
3	75	75	392	230
5	50	50	.....	.....
6	50	50	.....	.....
7	0	40	320	415
8	0	20	.....	.....
9	0	20	.....	.....
10	0	5	.....	.....
11	0	0	.....	.....

The pH values of the three samples were identical on the first day and all showed an equal, but slight, increase in acidity by the eighth day.

Trial 3 was similar to trial 2 except that the diluted semen was divided into 4 parts, the concentration of Amphyl in tube 4 being 0.5 per cent. The results on motility, shown in table 3, are similar to those obtained in the two preceding trials. There is some indication that a concentration 0.5 per cent Amphyl is too high for optimum effect. This is substantiated by the data in table 4, obtained from trial 4 in which the diluted semen contained 0.67 per cent Amphyl. The rate of decrease in motility was considerably greater than in the cases where a lesser concentration of Amphyl was used. These results indicate that the optimum concentration of Amphyl lies between 0.33 and 0.5 per cent.

The possibility that other disinfectants might have an effect similar to Amphyl on sperm in standard diluter was investigated in trial 5, in which Lysol and Zephiran were used in comparison with Amphyl. Lysol is a mixture of cresylic acid, neutral soap and glycerol. Zephiran is a mixture of high molecular weight alkyl-dimethyl-benzyl-ammonium chlorides. One volume of semen was diluted with 2 volumes of standard diluter and the

mixture was divided into 4 parts, each part consisting of 2 ml. of the diluted semen. Tube 1 served as the control, tube 2 contained one ml. of one per cent Amphyl, tube 3 contained one ml. of one per cent Lysol, and tube 4 contained one ml. of one per cent Zephiran. The results are presented in table 5.

The results presented in table 5 show that Lysol is highly toxic to sperm even though some motility persisted for three days. Zephiran did not exhibit much effect on sperm, although the rate of decrease in percentage of motility was slightly greater than in the control semen. Motility persisted

TABLE 2

Effect of Amphyl in different concentrations on the percentage of motile sperm, the persistence of sperm motility, the numbers of bacteria, and the pH of semen—trial 2

Days	Motility			Bacteria per ml. of undiluted semen			pH		
	Control	Treated*		Control	Treated*		Control	Treated*	
	1	2	3	1	2	3	1	2	3
	%	%	%	*thousands*					
1	95	95	95	356	339	275	6.4	6.4	6.4
2	90	85	85	.....	.....	.....	.....	.....	.....
3	80	80	75	.....	.....	.....	.....	.....	.....
4	50	50	50	.....	.....	.....	.....	.....	.....
5	20	40	40	.....	.....	.....	.....	.....	.....
6	5	30	30	.....	.....	.....	.....	.....	.....
7	5	20	20	.....	.....	.....	.....	.....	.....
8	0	20	20	.....	.....	.....	6.2	6.2	6.2
9	0	10	10	.....	.....	.....	.....	.....	.....
10	0	5	5	299	381	284	.....	.....	.....
11	0	5	5	.....	.....	.....	6.2	6.2	6.2
12	0	0	5	.....	236	279	.....	.....	.....
13	0	0	5	.....	.....	.....	.....	.....	.....
14	0	0	5	.....	.....	.....	.....	.....	.....
15	0	0	0	.....	.....	.....	.....	.....	.....

* Treated semen: tube 2 contained 0.2 per cent Amphyl; tube 3 contained 0.33 per cent.

for four days longer in the Amphyl-treated semen than in the control semen. The sperm in this sample of semen were unusually virile, as indicated by the persistence of motility in the control sample for ten days. Generally, motility persists for only five or six days.

An experiment was conducted to observe the effect of Amphyl on an organism other than bovine sperm. A pure culture of a hemolytic staphylococcus, which contained 500,000,000 organisms per ml., was treated with Amphyl in the presence of phosphate buffer alone, in the presence of egg yolk alone, and in the presence of the standard diluter. Ten ml. of each solution were sterilized, inoculated with 0.1 ml. of the staphylococcus culture,

and allowed to stand 30 minutes before being plated out in beef extract-blood (bovine) agar. The plates were incubated at 37° C. for 48 hours.

The results, presented in table 6, show that Amphyl destroyed all organisms in the presence of either the phosphate buffer alone or the egg yolk alone, whereas, in the presence of the standard diluter, many of the organ-

TABLE 3

Effect of Amphyl in different concentrations on the percentage of motile sperm, the persistence of sperm motility, and the numbers of bacteria in semen—trial 3

Days	Motility				Bacteria per ml. of undiluted semen			
	Control	Treated*			Control	Treated*		
	1	2	3	4	1	2	3	4
	%	%	%	%	*thousands*			
5 hours	95	95	90	90	.....	.....	.....	.....
1	90	95	90	90	375	320	437	376
2	80	80	80	70	.....	.....	.....	.....
3	80	70	70	70	.....	.....	.....	.....
4	50	60	50	50	.....	.....	.....	.....
5	30	60	50	50	.....	.....	.....	.....
6	0	45	40	20	.....	.....	.....	.....
7	0	15	40	30	331	372	440	319
8	0	0	20	20	.....	.....	.....	.....
9	0	0	35	20	.....	.....	.....	.....
10	0	0	30	15	.....	.....	.....	.....
11	0	0	30	15	.....	.....	.....	.....
12	0	0	30	15	.....	.....	.....	.....
13	0	0	30	10	.....	.....	.....	.....
14	0	0	5	5	.....	.....	.....	.....
15	0	0	0	0	.....	.....	.....	.....

* Treated semen: tube 2 contained 0.2 per cent Amphyl; tube 3 contained 0.33 per cent; tube 4 contained 0.5 per cent.

TABLE 4

Effect of Amphyl in high concentration (0.67%) on the percentage of motile sperm, the persistence of sperm motility, and the numbers of bacteria in semen—trial 4

Days	Motility	Bacteria per ml. of undiluted semen
	%	*thousands*
1	85	893
2	60	.....
3	40	.....
4	50	.....
5	30	.....
6	5	.....
7	0	.....
8	0	594

isms continued to grow. The number, however, was only about one per cent of the total which grew in the standard diluter medium in the absence of Amphyl. Tests showed, further, that organisms were still alive in this medium in the presence of one per cent Amphyl after seven days. These results agree well with the findings on bovine sperm.

TABLE 5

Effect of equal concentrations of Amphyl, Lysol, and Zephiran on the percentage of motile sperm and the persistence of sperm motility in semen diluted with standard diluter—trial 5

Days	Motility			
	Control semen	Treated semen		
		Amphyl	Lysol	Zephiran
	%	%	%	%
1	95	90	50	90
2	85	75	15	75
3	60	50	5	50
4	50	50	0	50
5	40	30	0	20
6	40	25	0	15
7	20	30	0	15
8	15	15	0	15
9	5	15	0	10
10	1	15	0	0
11	0	15	0	0
12	0	15	0	0
13	0	15	0	0
14	0	15	0	0
15	0	15	0	0

TABLE 6

Bactericidal effect of Amphyl on a hemolytic staphylococcus suspended in various media

Solutions	Bacteria per ml. *thousands*
10 ml. phosphate buffer	0
10 ml. phosphate buffer + 0.1 ml. culture	73,000
10 ml. phosphate buffer + 0.1 ml. culture + 1 ml. Amphyl	0
5 ml. egg yolk + 5 ml. water	0
5 ml. egg yolk + 5 ml. water + 0.1 ml. culture	173,000
5 ml. egg yolk + 5 ml. water + 0.1 ml. culture + 1 ml. Amphyl	0
10 ml. standard diluter	0
10 ml. standard diluter + 0.1 ml. culture	247,000
10 ml. standard diluter + 0.1 ml. culture + 1 ml. Amphyl	2,480

DISCUSSION

No completely satisfactory explanation of the behavior of bovine sperm or of the hemolytic staphylococcus in the presence of Amphyl can be offered. It seems possible that the toxicity of the disinfectant toward sperm and bacterial organisms is greatly reduced or completely nullified by the presence of the ingredients in the standard diluter. This conceivably could take place through some type of chemical combination, perhaps between Amphyl and egg yolk in the presence of phosphates. The increased longevity and motility of the sperm, however, cannot be explained on this basis.

SUMMARY

The motility of bovine sperm in undiluted semen ceased within 30 seconds after the addition of 0.5 per cent Amphyl, a disinfectant containing synthetic organic compounds.

Indications are that sperm, suspended in an egg yolk-phosphate buffer dilution medium containing from 0.2 to 0.5 per cent of Amphyl, maintained motility longer than that of sperm suspended in the same medium but containing no Amphyl.

Sperm suspended in phosphate buffer solution containing one per cent Amphyl soon lost all motility.

Sperm suspended in egg yolk-phosphate buffer solution containing Lysol lost all motility within three days, whereas, those suspended in the same medium but containing Zephiran remained motile for a period equal to that of the control sample.

Hemolytic staphylococci were completely destroyed in phosphate buffer and in egg yolk solution containing one per cent Amphyl; many of the staphylococci continued to grow after seven days when suspended in egg yolk-phosphate buffer solution containing one per cent Amphyl.

ACKNOWLEDGMENT

The research work reported in this paper was made possible through a fellowship supported by Lehn and Fink Products Corporation, Bloomfield, N. J. The authors gratefully acknowledge their indebtedness to this company.

Grateful acknowledgment also is made to Mr. C. E. Shuart and Dr. Donald Masters, New Jersey Dairy Research Station, Sussex, N. J., for their aid in collecting and handling the samples of semen.

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THE VITAMIN A VALUE OF ROQUEFORT TYPE CHEESE*

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INTRODUCTION

Several factors have been reported to have an effect on the vitamin A value of cheese. Hathaway and Davis (6) in a study of twenty different kinds of cheese found considerable variation in vitamin A content of any given kind of cheese. They reported that the vitamin A value was influenced by the vitamin A potency of the milk used, the amount of butterfat in the milk and the methods of processing, packaging and storage. Carotenoids have been shown to be present in microorganisms and the ability of certain microorganisms to synthesize carotene has been demonstrated (1, 9). It was considered possible that the organisms used in the manufacture of Roquefort cheese also might have the capacity to produce carotene or vitamin A. Therefore, the following investigation was planned to determine the vitamin A value of cheese ripened with Roquefort mold, similar cheese without mold growth and the unripened frozen curd.

EXPERIMENTAL

Preparation of cheese: The cheeses were all made on September 10, 1940, from the same lot of raw milk in the same vat. The method of manufacture used was a modification of two well known processes (5, 7) for the making of blue cheese. The modifications were to obtain the cheese in cylinders of $3\frac{1}{8}''$ with the desired moisture content. Just before the curd was put in the cylinders it was inoculated with a mold powder prepared from a culture of *Penicillium roqueforti*, Culture 33D (2, 3). The next day the cylinders of curd were cut into pieces $1\frac{1}{2}''$ high and brine salted for 2 hours in a saturated brine solution. At this stage the curd was divided into three groups:

(a) Frozen Roquefort type curd. The salted curd was vacuum packed in #2 short one-half pound flat Columbia River sized re-enamel cans. This curd was prevented from ripening into cheese by holding it at 0° F. or lower until used.

(b) Roquefort type cheese, ripened without mold growth. This lot was vacuum canned exactly the same as lot (a), thus inhibiting mold growth, but was allowed to ripen in the vacuum sealed cans at the same temperature as lot (c).

(c) Roquefort type cheese, ripened with mold growth. This lot was

Received for publication June 10, 1942.

* Published as Scientific Paper No. 526, College of Agriculture and Agricultural Experiment Station, State College of Washington.

placed in the cans with lids loosely placed on. Both lots (b) and (c) were then held in a gas controlled chamber (4) at a temperature of 50° F. until October 29, 1940, when lot (c) had developed a good growth of *P. roqueforti*. The cans of lot (c) were then vacuum sealed. Both lots (b) and (c) were then placed in cool storage (30° F. to 40° F.) until used.

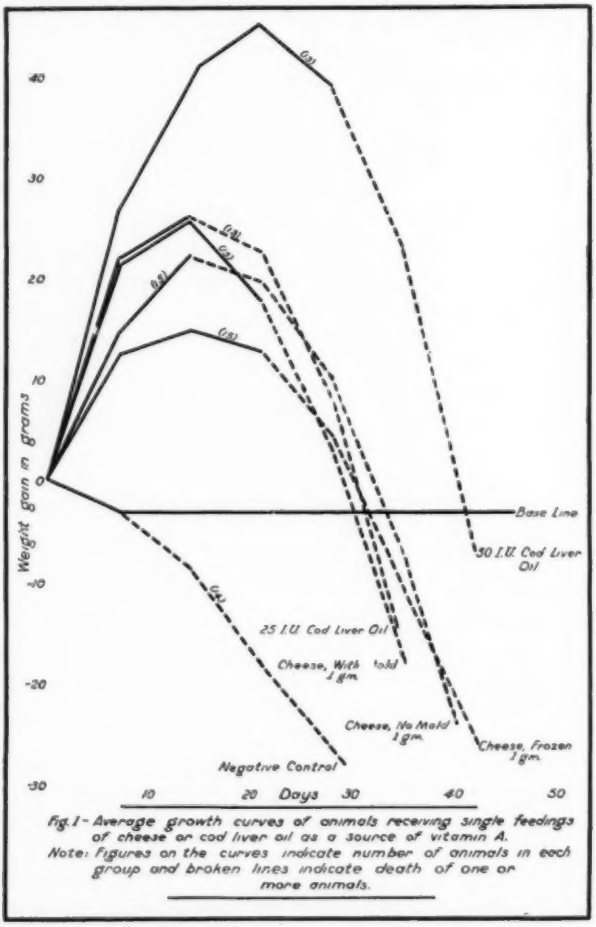

Fig. 1 - Average growth curves of animals receiving single feedings of cheese or cod liver oil as a source of vitamin A. Note: Figures on the curves indicate number of animals in each group and broken lines indicate death of one or more animals.

Method of assay: Vitamin A assays were carried out by the single-feeding method of Sherman and Todhunter (8). Eighty-five albino rats, 40 to 50 grams in weight, and 21 to 28 days of age, and with low initial reserves of vitamin A were prepared for the assay according to the directions of the U. S. Pharmacopoeia XI. The basal diet consisted of: casein (heat-treated)

18 per cent; cornstarch 65 per cent; yeast 8 per cent; salt mixture (Osborne and Mendel) 4 per cent; Wesson oil 5 per cent. Viosterol, three drops per week, was given orally throughout the experiment. The rats were depleted of their reserves of vitamin A in 25 to 36 days and were allotted to one of six groups which were equalized for sex, litter, weight and length of time of depletion. The groups were as follows: (a) Frozen Roquefort type curd, one gram, (b) Roquefort type cheese, ripened without mold growth, one gram, (c) Roquefort type cheese, ripened with mold growth, one gram, (d) basal diet only, (e) reference cod liver oil, 25 I.U., (f) reference cod liver oil, 50 I.U. Single feedings of these supplements were given and the animals were allowed basal diet *ad libitum* until death. Growth curves were drawn for all animals and the area under the curve, using the base line as described by Sherman and Todhunter (8) was measured with a planimeter.

RESULTS AND DISCUSSION

The data are summarized in table 1 and figure 1. In order to determine whether the areas under the curves were significantly different, the data were

TABLE 1

Vitamin A value of Roquefort type cheese

Supplement	Moisture per cent	Number of animals		Area under curve	Vitamin A value (I.U. per gm.)
		M	F		
(a) Frozen Roquefort type curd, 1 gm.	45.52	8	7	3.91	25
(b) Roquefort type cheese ripened without mold growth, 1 gm.	44.94	8	7	5.48	25
(c) Roquefort type cheese curd ripened with mold growth, 1 gm.	41.67	8	7	5.51	25
(d) Basal diet					
(e) Reference cod liver oil, 25 I.U.		5	8	6.20	1700
(f) Reference cod liver oil, 50 I.U.		6	7	13.37	1700

treated statistically by the analysis of variance method. The areas for the curves of the three samples of cheese were not significantly different from each other nor from that obtained when 25 I.U. of vitamin A were fed as reference cod liver oil. Therefore, it is concluded that the vitamin A value of each of these samples of cheese was approximately 25 I.U. per gram, and that the Roquefort organism, Culture 33D, was without influence on the vitamin A value of the cheese under these conditions. Hathaway and Davis (6) reported values of 40 I.U. of vitamin A per gram of Roquefort cheese.

The area under the curve for the group receiving 50 I.U. of vitamin A as reference oil was almost exactly twice that of the group receiving 25 I.U. of vitamin A and the difference in area was found to be statistically significant.

This offers confirmatory evidence that the area under the curve is proportional to the amount of vitamin A fed and that the method of single feeding is reliable for vitamin A measurements.

SUMMARY

The vitamin A value of Roquefort type cheese with and without mold development was determined by bioassay using the method of single feeding. The vitamin A content was the same for all samples of cheese, approximately 25 I.U. per gram. No evidence was obtained that mold growth, by Culture 33D, in Roquefort type cheese increased the vitamin A value.

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OXIDIZED FLAVOR IN MILK. XI. ASCORBIC ACID, GLUTATHIONE, AND HYDROGEN PEROXIDE AS MECHANISMS FOR THE PRODUCTION OF OXIDIZED FLAVOR*

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Hand and Sharp (11) recently proposed that changes in the flavor of milk might be produced by certain pigments, vitamins, and enzymes. The riboflavin system was mentioned as was also the oxidation of ascorbic acid with cucumber "oxidase." They found that other catalysts of ascorbic acid oxidation produce hydrogen peroxide. Thurston, Brown, and Dustman (24) reported that oxidized flavor in milk was caused by the oxidative breakdown of lecithin in milk. The term lecithin was used as inclusive for the phospholipids. Lecithin and cephalin have been shown to be easily oxidized (5). From this it would seem that the mechanism for the development of oxidized flavor in milk might be the oxidation of ascorbic acid with the production of hydrogen peroxide which then oxidizes the phospholipids, liberating the compounds which give the oxidized flavor.

In studying the oxidation of phospholipids in relation to cancer, Deutsch, Kline, and Rusch (9) found by manometric technique that the reaction was catalyzed by the presence of ascorbic acid at a pH of 4.0. In a further study Rusch and Kline (21) found that the oxidation of various phospholipid preparations was catalyzed by ascorbic acid, glutathione, and cysteine at pH 3.5 but that these substances were inactive at pH 7.4. Thiamine, riboflavin, pyridoxine, and methylene blue catalyzed the oxidation at pH 7.4 but not at pH 3.5. They found that certain carcinogenic hydrocarbons and hydroquinone, catechol, carotene, and cholesterol, as well as other compounds, inhibited the oxidation. Most of the compounds catalyzing the reaction and some of the inhibitors are found in milk.

Barron, Barron and Klemperer (1) studied the oxidation of ascorbic acid catalyzed by copper. Using the manometric technique they found that the amount of ascorbic acid oxidized was proportional to the copper content of milk. Orange juice, tomato juice, and grapefruit juice also showed oxidation of ascorbic acid with copper at higher acidities.

Stotz, Harrer, and King (22) made a startling discovery when they attributed the catalytic activity of "ascorbic acid oxidase" from cauliflower and squash juices to the copper present in combination with protein. A

Received for publication June 10, 1942.

* Published with the approval of the Director of the West Virginia Agricultural Experiment Station as Scientific Paper No. 288.

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mixture of copper and albumin was found to have the same characteristics as the "enzymes" in relation to oxidation of ascorbic acid, optimum pH, and inactivation by heat. In a further study McCarthy, Green, and King (16) compared the copper protein complexes and cucumber "oxidase" with the similar system potato oxidase on catechol. The potato system was found to be different from the cucumber system, but the cucumber "oxidase" was similar to the copper protein complex. Meiklejohn and Stewart (17) compared the cucumber "oxidase" with a copper protein complex and found that the cucumber oxidase was more active. They did, however, find that complete removal of copper inactivated the cucumber enzyme.

Hand and Griesen (10) made a study of different catalysts for the oxidation of ascorbic acid. Using Barcroft manometers to measure the oxygen consumption they found that cucumber "oxidase" caused the absorption of one atom of oxygen per molecule of ascorbic acid; copper at various pH values caused absorption of between one and two atoms of oxygen; riboflavin and light caused absorption of two atoms of oxygen at pH 4 to 5. They explained that the reason for combination of one molecule of ascorbic acid with more than one atom of oxygen was the intermediate formation of hydrogen peroxide. They stated: "If the the hydrogen peroxide oxidizes ascorbic acid or is decomposed by catalase the number of oxygen atoms equals one; but if hydrogen peroxide accumulates in the solution or oxidizes dehydroascorbic acid the number of oxygen atoms equals two."

In a review of the oxidase systems of higher plants, Boswell and Whiting (4) found that the ascorbic acid oxidase, dihydroxy maleic acid oxidase, and catechol oxidase all belonged to the same system. The reason for this was the same grouping in the substrates, the $\begin{matrix}-C-OH\\ \|\\ -C-OH\end{matrix}$ group. With all three compounds hydrogen peroxide was formed when the right catalysts were used for the oxidation. Barron, De Meio, and Klemperer (2) explained the formation of hydrogen peroxide by the fact that ascorbic acid was oxidized by the cupric ion forming dehydroascorbic acid and cuprous ion. The cuprous ion was then oxidized by oxygen of the air to the cupric ion producing hydrogen peroxide. Dekker and Dickinson (8) gave a similar explanation for the production of hydrogen peroxide by copper catalytic oxidation of the ascorbic acid.

Robertson, Ropes and Bauer (19) studied a system somewhat similar to milk in the degradation of mucins and polysaccharides by ascorbic acid and hydrogen peroxide. That hydrogen peroxide was the cause of the mucin degradation was proved by getting no reaction when catalase was added to the system compared to a degradation of mucin when it was treated with ascorbic acid in copper sulphate solution.

Corbett and Tracy (6) reported an acceleration of oxidized flavor in some cases and protection in other cases with ascorbic acid in milk. Dahle

and Palmer (7) as well as a number of others have found ascorbic acid to protect milk from oxidized flavor.

Hopkins (13) studied the influence of glutathione on fats and proteins. At pH 3 to 4 the fatty acids, linoleic and linolenic, were oxidized by glutathione and oxygen. At pH 7.4 to 7.6 he found that half the oxygen uptake was used for the oxidation of the sulfhydryl group of glutathione and half for the oxidation of lecithin. Tait and King (23) in studying the oxidation of lecithin in the presence of glutathione found that the oxygen uptake of lecithin was about four times that which would be expected from a consideration of its iodine number in relation to the iodine number of its constituent fatty acids and their oxygen uptake. Hopkins and Morgan (14) studied the relationship of glutathione with ascorbic acid. They found that as long as glutathione was present in the unoxidized form, ascorbic acid was completely protected from oxidation, but after the glutathione was oxidized the ascorbic acid oxidized normally. Rosenthal and Voegtlin (20) found that copper ions oxidized reduced glutathione only to the oxidized form but they oxidized cysteine, which has a similar sulfhydryl group, from the reduced form to the break down constituents of ammonia, carbon dioxide and sulphuric acid.

Many theories can be postulated on the mechanism of oxidized flavor in milk, but some method of testing the theories must be found and in applying them to milk it is necessary to have sufficient sample so that the flavor may be determined by taste. In the papers reviewed most of the investigators have used manometers to measure the oxygen absorbed in order to determine the rate of oxidation. The use of milk is complicated by the number of interfering substances. Palmer and Wiese (18) washed cream from five to eight times to remove the milk plasma solids. This left only the fat globules composed of butter fat and fat globule membrane, the latter being a mixture of protein and phospholipids. If it is the phospholipids that give the oxidized flavor, they should be easily oxidized in washed cream and this could be used to test the theories. Accordingly, the following experiment was planned and conducted.

EXPERIMENTAL

For use in this experiment, cows whose milks were susceptible to oxidized flavor were selected and the milk from these animals was used throughout the experiment.

Samples of milk were collected from these cows each morning and placed in a ten gallon aluminum can. The milk was then pasteurized by immersion of the can in a vat fitted with steam coils and a cold water inlet. The temperature of the milk was raised to 144° F. $\pm$ 1° F. and held for 30 minutes. Following pasteurization a quart sample of the milk was removed and used as a control. The remainder of the milk was then separated and the

cream was washed by dilution with a volume of hot water equal to the volume of the skim milk removed. This was repeated five times so that practically all of the milk plasma solids were removed. In order to control the pH a buffer (6.8) was used in certain cases. This buffer was heated to 140–150° F. and added to the washed cream and the cream was separated a sixth time. One examination of the washed cream revealed only 0.25 per cent protein indicating practically complete protein removal. The washed cream thus obtained was then ready for experimental treatment.

The control sample of milk was divided into four half-pints. One of these was used as a control with no added copper while the others were contaminated with 0.5, 1.0, and 1.5 p.p.m. of copper, respectively, added from a copper sulphate solution. The cream was divided into two lots. Each lot was then divided and contaminated with copper in the same way as the milk. In addition to the added copper, in the second lot 50 mg. of ascorbic acid was added to each half-pint sample, which is equivalent to 210 mg. per liter. All samples were then stored at 40° F. $\pm$ 5° F. for three days, after which they were scored by at least three judges familiar with oxidized flavor. Since the oxidized flavor developed in the washed cream was so much stronger than that developed in the control milk, no attempt was made to classify its intensity. The results of this experiment are shown in table 1. These results show quite clearly that copper added to washed cream will not catalyze the development of oxidized flavor. In none of the five trials was there any trace of oxidized flavor developed. In the trials in which 210 mg. per liter of ascorbic acid were added to the washed cream prior to contamination with copper, a strong oxidized flavor developed. In most cases where the ascorbic acid was added to the control sample of washed cream containing no added copper the oxidized flavor likewise developed.

Since ascorbic acid has been reported to protect milk against the development of oxidized flavor it seemed desirable to study the effect of various concentrations of ascorbic acid on the susceptibility of washed cream. Accordingly, washed cream was fortified with various cncentrations of ascorbic acid and contaminated with copper as in the preceding experiment. The results are shown in table 2. An examination of these results shows that when washed cream was fortified with 630 mg. per liter of ascorbic acid no oxidized flavor developed. The addition of buffer to the washed cream to control the pH of the reaction did not change the results. At concentrations of ascorbic acid of 21 mg. per liter the development of oxidized flavor was erratic. However, concentrations of from 40 to 100 milligrams per liter gave the strongest development of the flavor.

In the previous experiments, it was observed that the oxidized flavor developed by addition of ascorbic acid to washed cream was many times as strong as that produced in susceptible milk. In many instances it was necessary to use normal milk and dilute the highly flavored washed cream so

TABLE 1

Relationship of ascorbic acid to oxidized flavor in washed cream

Date	Control milk				Washed cream								Buffered or unbuffered
					No added ascorbic acid				210 mg./l. ascorbic acid				
	p.p.m. copper				p.p.m. copper				p.p.m. copper				
	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5	
2/2/42	–*	2*	3*	3*	.....	.....	.....	.....	ox†	ox	ox	ox	Unbuffered
2/4/42	–	2	3	3	.....	.....	.....	.....	ox	ox	ox	ox	"
2/13/42	–	1	1	1	–	–	–	–	ox	ox	ox	ox	"
2/14/42	–	1	1	1	–	–	–	–	.....	.....	.....	.....	Buffered
2/16/42	–	1	1	1	–	–	–	–	ox	ox	ox	ox	Unbuffered
2/17/42	–	1	1	2	–	–	–	–	ox	ox	ox	ox	Buffered
2/19/42	–	1	1	1	.....	.....	.....	.....	ox	ox	ox	ox	"
2/19/42	–	1	1	1	.....	.....	.....	.....	acid	ox	ox	ox	Unbuffered
2/24/42	–	1	2	2	–	–	–	–	.....	.....	.....	.....	Unbuffered
2/25/42	–	1	1	1	.....	.....	.....	.....	ox	ox	ox	ox	Unbuffered

* Intensity of oxidized flavor: – no oxidized flavor; ? doubtful; 1 slight oxidized flavor; 2 moderate oxidized flavor; 3 pronounced oxidized flavor; and 4 very pronounced oxidized flavor.

† Oxidized flavor.

TABLE 2

Relationship of concentration of ascorbic acid to oxidized flavor in washed cream

Date	Control milk				Washed cream																Buffered or unbuffered
					630 mg./l. ascorbic acid				210 mg./l. ascorbic acid				105 mg./l. ascorbic acid				42 mg./l. ascorbic acid				21 mg./l. ascorbic acid				
	p.p.m. copper				p.p.m. copper				p.p.m. copper				p.p.m. copper				p.p.m. copper				p.p.m. copper				
	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5	
2/18/42	–*	2*	2*	3*	.....	.....	.....	.....	ox*	ox	ox	ox	ox	ox	ox	ox	ox	ox	ox	ox	ox	ox	ox	ox	Buffered
2/23/42	–	1	2	2	.....	.....	.....	.....	ox	ox	ox	ox	ox	ox	ox	ox	ox	ox	ox	ox	ox	ox	ox	ox	"
2/25/42	–	1	1	1	.....	.....	.....	.....	ox	ox	ox	ox	ox	ox	ox	ox	ox	ox	ox	ox	.....	.....	.....	.....	Unbuffered
2/27/42	–	1	1	2	.....	.....	.....	.....	.....	.....	.....	.....	ox	ox	ox	ox	ox	ox	ox	ox	ox	ox	ox	ox	"
3/13/42	–	1	2	2	.....	.....	.....	.....	–	ox	ox	ox	ox	ox	ox	ox	ox	ox	ox	ox	.....	.....	.....	.....	"
3/16/42	–	1	1	1	–	–	–	–	–	ox	ox	ox	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	"
					–	–	–	–	ox	ox	ox	ox	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	Buffered
3/17/42	–	2	2	3	–	–	–	–	–	ox	ox	ox	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	Unbuffered
					–	–	–	–	ox	ox	ox	ox	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	Buffered
3/19/42	–	1	2	2	–	–	–	–	–	ox	ox	ox	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	Buffered
3/24/42	–	1	1	1	–	–	–	–	–	ox	ox	ox	ox	ox	ox	ox	.....	.....	.....	.....	.....	.....	.....	.....	Unbuffered
3/25/42	–	1	1	1	.....	.....	.....	.....	ox	ox	ox	ox	ox	ox	ox	ox	ox	ox	ox	ox	–	–	–	–	"
3/26/42	–	1	1	1	.....	.....	.....	.....	ox	ox	ox	ox	ox	ox	ox	ox	ox	ox	ox	ox	.....	.....	.....	.....	"
3/27/42	–	1	1	1	.....	.....	.....	.....	ox	ox	ox	ox	ox	ox	ox	ox	–	?	?	–	.....	.....	.....	.....	"

* See table 1, footnote.

TABLE 3

The relationship of ascorbic acid to oxidized flavor in washed cream from non-susceptible milk

Date	Control milk				Washed cream																Buffered or unbuffered
					210 mg./l. ascorbic acid				105 mg./l. ascorbic acid				42 mg./l. ascorbic acid				21 mg./l. ascorbic acid				
	p.p.m. copper				p.p.m. copper				p.p.m. copper				p.p.m. copper				p.p.m. copper				
	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5	
4/17/42	–*	–	–	–	!	ox*	ox	ox	ox	ox	ox	ox	!	ox	ox	ox	.....	.....	.....	.....	Unbuffered
4/18/42	–	–	–	–	–	ox	ox	ox	ox	ox	ox	ox	–	ox	ox	ox	–	–	–	–	"
4/20/42	–	–	–	–	–	ox	ox	ox	ox	ox	ox	ox	ox	ox	ox	ox	–	–	!	!	"
4/21/42	–	–	–	–	–	–	ox	ox	ox	ox	ox	ox	ox	ox	ox	ox	–	–	–	–	"
4/22/42	–	–	–	–	–	ox	ox	ox	ox	ox	ox	ox	ox	ox	ox	ox	–	–	–	!	"

* See table 1, footnote.

as to obtain a typical oxidized flavor. Since the flavor developed in washed cream was so much stronger than that developed in normal milk it seemed desirable to study the effect of ascorbic acid on non-susceptible milk. Several attempts were made to select cows which gave non-susceptible milk, but during the dry feeding period when sufficient quantity of milk was selected there was usually a suggestion of oxidized flavor after the three-day storage period. Accordingly, several cows were selected and placed on pasture during the day. After about one week of early spring pasture the morning milk from these cows was tested and found to be non-susceptible. This milk was then used in obtaining the data in table 3. The results obtained in this study show that an oxidized flavor can be developed in washed cream from normally non-susceptible milk. An examination of these data reveals quite clearly that the material rendering milk non-susceptible to oxidized flavor, when contaminated with copper, is carried in the water phase of the milk. These results are in agreement with the observations of Krukovsky (15).

Since ascorbic acid has been shown to catalyze the oxidation of phospholipids and produce an oxidized flavor in washed cream, it seemed advisable to determine if several of the other materials suggested by Rusch and Kline (21) as catalysts for this reaction, might likewise catalyze the development of oxidized flavor. Accordingly, several of these materials were used in various concentrations. The results of this study are shown in table 4. An examination of these data reveals that thiamine, pyridoxine, riboflavin, and cysteine, do not catalyze the development of oxidized flavor in washed cream even in the presence of copper. The data obtained with riboflavin were rather limited but when increased concentrations were used they caused the development of a bitter flavor which would have masked any oxidixed flavor present. The work of Hand and Griesen (10) suggests a role for riboflavin through the oxidation of the ascorbic acid. However, because of the bitter flavor developed this could not be checked by taste. Cysteine was tried at several different concentrations but at higher concentrations its flavor was such as to mask any oxidized flavor developed.

Glutathione in the presence of copper caused the development of an oxidized flavor. It was noted that as the concentration of the copper increased, the intensity of the oxidized flavor developed also increased.

DISCUSSION AND RESULTS

The results obtained in this experiment indicate that either ascorbic acid or glutathione, or both, are probably involved in the mechanism whereby an oxidized flavor is developed in milk. Corbett and Tracy (6) reported that in copper contaminated milk the ascorbic acid first retarded the development of the oxidized flavor and then after a certain point was reached, the development of the oxidized flavor was accelerated. Results obtained in the present study show that high concentrations of ascorbic

TABLE 4

The relationship of certain compounds to oxidized flavor in washed cream

Date	Control milk				Washed cream																Buffered or unbuffered
					210 mg./l. thiamine				105 mg./l. pyridoxine				4 mg./l. riboflavin				10 mg./l. cysteine				105 mg./l. glutathione				
	p.p.m. copper				p.p.m. copper				p.p.m. copper				p.p.m. copper				p.p.m. copper				p.p.m. copper				
	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5	
2/6/42	–	2*	3*	3*	–	–	–	–																	Unbuffered
2/12/42	–	2	2	3	–	–	–	–																	Buffered
3/14/42	–	1	1	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–					Unbuffered
2/16/42	–	1	2	3					–	–	–	–													"
3/16/42	–	1	1	1					–	–	–	–													"
2/23/42	–	1	2	2													–	–	–	–					Buffered
3/12/42	–	1	1	2																	–	ox	ox	ox	Unbuffered
3/17/42	–	2	2	3																	–	–	ox	ox	"
3/24/42	–	1	1	1																	–	ox	ox	ox	"

* See table 1, footnote.

TABLE 5

The effect of hydrogen peroxide on the development of oxidized flavor in washed cream

Date	Control milk				Washed cream											
					Containing 0.01% hydrogen peroxide				Containing 0.05% hydrogen peroxide				Containing 0.1% hydrogen peroxide			
	p.p.m. copper				p.p.m. copper				p.p.m. copper				p.p.m. copper			
	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5
2/21/42	–	1*	2	2	–	?*	?	?	.....	.....	.....	.....	–	ox	ox	ox
3/2/42	–	1	1	2	–	–	–	–	–	–	–	–	–	–	ox	ox
3/3/42	–	1	1	2	–	–	–	ox	–	ox	ox	ox	–	ox	ox	ox
3/6/42	–	?	1	2	–	ox	ox	ox	–	ox	ox	ox	–	ox	ox	ox
3/9/42	–	1	2	2	–	?	ox	ox	–	ox	ox	ox	–	ox	ox	ox
3/10/42	–	1	1	1	–	ox	ox	ox	–	ox	ox	ox	–	ox	ox	ox
					Increasing → Intensity				Increasing → Intensity				Increasing → Intensity			

* See table 1, footnote.

acid protect cream against oxidized flavor while lower concentrations cause the flavor to develop. The use of a buffer to control the pH did not affect these results.

Both glutathione and ascorbic acid catalyze the development of oxidized flavor. Any theory as to the reaction whereby this oxidation is brought about should be applicable to both substances. A review of the literature published on the oxidation of ascorbic acid reveals that the oxidation of these two substances may have certain similarities. The oxidation of ascorbic acid by copper to dehydroascorbic acid has been reported to liberate hydrogen peroxide (2, 8, 10, 19).[3] Likewise, it has been postulated (25) that hydrogen peroxide is liberated when glutathione is oxidized with copper. The references just cited indicate that cupric copper is reduced by either ascorbic acid or glutathione to the cuprous form. The cuprous form is then oxidixed by molecular oxygen to form cupric copper and hydrogen peroxide.

Since hydrogen peroxide is a strong oxidizing substance it may be possible that it reacts with the phospholipids to oxidize them and thus bring about the development of an oxidized flavor. This particularly seems true since it was impossible to develop an oxidized flavor in washed cream with copper alone. Such a theory suggested the direct addition of hydrogen peroxide to washed cream. This was tried with the results shown in table 5. An examination of these data shows that hydrogen peroxide in proper concentration with copper as a catalyst does produce an oxidized flavor. As the rate of copper contamination increased the intensity of the oxidized flavor developed likewise increased. The role of copper in this oxidation with hydrogen peroxide is not known.

Although the development of oxidized flavor by means of hydrogen peroxide does not give positive proof that hydrogen peroxide is liberated during the reaction in which ascorbic acid or glutathione is oxidized in the development of the flavor, it does give indirect evidence that such a mechanism may exist and may be responsible when an oxidized flavor is developed.

CONCLUSIONS

1. Washed cream from susceptible milk does not develop an oxidized flavor when contaminated with copper and stored for three days.
2. Washed cream fortified with ascorbic acid (40 to 200 mg. per liter) and contaminated with copper develops a strong oxidized flavor.
3. Washed cream containing added glutathione develops an oxidized flavor when contaminated with copper.
4. Washed cream from non-susceptible milk develops an oxidized flavor when contaminated with copper and fortified with ascorbic acid.

[3] Since this manuscript was prepared, Steinman and Dawson have submitted additional proof for the production of hydrogen peroxide when ascorbic acid is oxidized by the Cupric ion. Jour. Amer. Chem. Soc., 64: 1212, 1942.

5. The addition of 0.10 per cent hydrogen peroxide to washed cream contaminated with copper causes the development of oxidized flavor.

6. Under the conditions of this experiment, thiamine, pyridoxine, riboflavin, and cysteine in the presence of copper do not cause the development of oxidized flavor in washed cream.

ACKNOWLEDGMENT

The authors are indebted to Dr. H. K. Johnston, Department of Dairy Husbandry, for assistance in scoring the experimental samples of milk and washed cream; and to Dr. R. B. Dustman, Department of Agricultural Chemistry, for his assistance and constructive criticisms in the preparation of this paper.

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OXIDIZED FLAVOR IN MILK. XII. FURTHER STUDIES OF ASCORBIC ACID MECHANISMS IN THE PRODUCTION OF OXIDIZED FLAVOR IN MILK*

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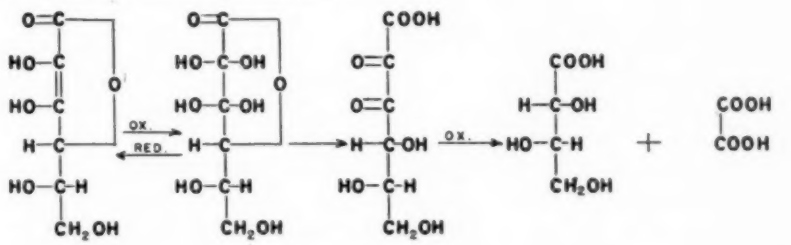
The role of ascorbic acid in the production of oxidized flavor in washed cream has been reported by Olson and Brown (8). The mechanism was ascribed to the following reactions: (a) reduction of cupric copper by ascorbic acid, which was oxidized to dehydroascorbic acid, (b) oxidation of the cuprous ion to the cupric ion by oxygen from the air with the liberation of hydrogen peroxide, and (c) oxidation of the phospholipids in the fat globule membrane of the cream by the hydrogen peroxide, producing the oxidized flavor.

Many studies have been made of the oxidation of ascorbic acid to dehydroascorbic acid, but very little information is available on the oxidation of dehydroascorbic acid.

Stotz, Harrer, and King (10) found difficulty in explaining the absorption of oxygen by copper-catalyzed ascorbic acid at a pH of 7.4. They found that about twice as much oxygen was consumed as was indicated by the indophenol titration.

Borsook *et al.* (1) made a study of the oxidation of ascorbic acid and proposed the following reactions:



Received for publication June 10, 1942.

* Published with the approval of the director of the West Virginia Agricultural Experiment Station as Scientific Paper No. 290.

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The first reaction was found to be reversible. At a pH below 4.0 the oxidation was found to stop at the dehydro form. Above pH 4.0 an irreversible nonoxidative rearrangement changed the dehydroascorbic acid to diketogulonic acid. That dehydroascorbic acid undergoes a rearrangement has been shown by a change in optical rotation by Herbert *et al.* (5). By the use of dyes, Borsook *et al.* (1) were able to determine the oxidation-reduction potential of the diketogulonic acid; they found it to be more reducing and a stronger acid than the reduced ascorbic acid. Between pH 4.0 and 9.0 the diketogulonic acid could be oxidized to oxalic acid and l-threonic acid. At a pH above 9.0 they believed the threonic acid was oxidized to tartaric acid.

Steinman and Dawson (9) studied the mechanism for the oxidation of ascorbic acid. They found that when ascorbic acid was oxidized by the cupric ion, hydrogen peroxide was evolved, but when the reaction was catalyzed by the enzyme oxidase (copper-proteinate) no hydrogen peroxide was evolved.

At the pH of milk the reactions whereby ascorbic acid and diketogulonic are oxidized are both possible. Whether or not the diketogulonic acid can be oxidized by copper with the production of hydrogen peroxide as in the reduced ascorbic acid is not known, but the fact that the diketogulonic acid is more reducing than ascorbic acid would seem to indicate this possibility. If the development of oxidized flavor in milk follows the mechanism outlined there appears to be little possibility for the action of an enzyme, although such an enzyme has already been reported.

Kende (7) reported that milk heated to 80–85° C. did not develop an oxidized flavor and attributed this to the inactivation of the enzyme "oleinase." Kende (7) and later Chilson (2) and Dahle and Palmer (3) found the heat labile substance responsible for the oxidized flavor to be associated with the milk plasma. Gould and Sommer (4) and Josephson and Doan (6) found that sulfhydryl compounds formed when milk was heated to high temperatures, acted as antioxidants and inhibited the development of oxidized flavor. Gould and Sommer (4) stated "A further proof that the enzyme theory may not be an entirely satisfactory explanation relative to heat prevention of oxidized flavor is that the same temperatures which inhibited the flavor when the copper was added prior to the heating process were of no benefit when the copper was added following heating." These results make questionable the presence of an enzyme in the development of oxidized flavor. Since it is possible to develop an oxidized flavor in washed cream; and since washed cream would contain practically no sulfhydryl groups after washing, it seemed advisable to determine if high temperature pasteurization would inhibit the development of oxidized flavor in washed cream. Accordingly, this factor was likewise studied.

EXPERIMENTAL

Milk samples were obtained from cows whose milks were susceptible to oxidized flavor development and washed cream and control milk samples for use in this experiment prepared as previously described (8).

The control sample of milk was divided into four half-pints. One of these was used as a control with no added copper while the others were contaminated with 0.5, 1.0, and 1.5 p.p.m. of copper, respectively, added from a copper sulphate solution. The washed cream was divided into three lots. Each lot was then divided and contaminated with copper in the same way as the milk. In addition to the added copper, lot one was fortified with 50 mg. of ascorbic acid per half-pint sample, which is equivalent to 210 mg. per liter. Lot two contained the same amount of ascorbic acid and sufficient KI to make a 0.1 per cent solution of the cream. Lot three was fortified with 210 mg. of dehydroascorbic acid in the same manner as lot two. The dehydroascorbic acid was prepared by oxidation of ascorbic acid with iodine solution after the method of Herbert *et al.* (5). The potassium iodide was added to lot two as a control to show the effect of potassium iodide on ascorbic acid. All samples were then stored at 40° F. ± 5° for three days, after which they were scored by at least three judges familiar with oxidized flavor. Since the oxidized flavor which developed in the washed cream was so much stronger than that developed in the control milk, no attempt was made to classify its intensity. The results of this experiment are shown in table 1. An examination of the data reveals that dehydroascorbic acid as well as the reduced ascorbic acid promotes an oxidized flavor. However, the dehydroascorbic acid produces an oxidized flavor only in a buffered solution whereas the reduced ascorbic acid will promote the flavor in either buffered or unbuffered washed cream. The addition of 0.1 per cent KI prevented the development of an oxidized flavor in the washed cream when 210 mg. per liter of ascorbic acid was present. Buffering had no effect on this reaction.

It seemed desirable to determine whether or not the concentration of dehydroascorbic acid had any appreciable effect on the development of the oxidized flavor in washed cream. Accordingly, several concentrations were selected and set up in the same manner as those reported in table 1. The results are reported in table 2. An examination of these data reveals clearly that concentrations of dehydroascorbic acid from 210 to 21 mg. per liter will, in most cases, cause the development of an oxidized flavor in washed cream contaminated with copper. All trials were made on buffered creams.

An examination of the data in table 1 reveals that an oxidized flavor was not developed in washed cream when it contained 0.1 per cent KI. Since the strongest oxidized flavors were developed at concentrations of 40 to 100 mg. of ascorbic acid per liter it seemed advisable to determine if the effect of potassium iodide in protecting against this flavor held throughout this

TABLE 1

The relationship of ascorbic acid and dehydroa scorbic acid to oxidized flavor in washed cream

Date	Control milk				Washed cream												Buffered or unbuffered
					210 mgm./l. ascorbic acid				210 mgm./l. ascorbic acid + 0.1% KI				210 mgm./l. dehydroascorbic acid + 0.1% KI				
	p.p.m. copper				p.p.m. copper				p.p.m. copper				p.p.m. copper				
	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5	
2/2/42	–*	2*	3*	3*	ox†	ox	ox	ox	.....	.....	.....	.....	–	–	–	–	Unbuffered
2/4/42	–	2	3	3	ox	ox	ox	ox	.....	.....	.....	.....	–	–	–	–	"
2/10/42	–	2	3	3	.....	.....	.....	.....	–	–	–	–	ox	ox	ox	ox	Buffered
2/14/42	–	1	1	1	.....	.....	.....	.....	–	–	–	–	ox	ox	ox	–	"
2/17/42	–	1	1	2	ox	ox	ox	ox	–	–	–	–	ox	ox	ox	ox	"
2/19/42	–	1	1	1	ox	ox	ox	ox	–	–	–	–	ox	ox	ox	ox	"
2/19/42	–	1	1	1	–	ox	ox	ox	–	–	–	–	–	–	–	–	Unbuffered
2/21/42	–	1	2	2	ox	ox	ox	ox	–	–	–	–	.....	.....	.....	.....	"
2/24/42	–	1	2	2	.....	.....	.....	.....	–	–	–	–	–	–	–	–	"

* Intensity of oxidized flavor: – no oxidized flavor; ? doubtful; 1 slight oxidized flavor; 2 moderate oxidized flavor; 3 pronounced oxidized flavor; and 4 very pronounced oxidized flavor. † Oxidized flavor.

TABLE 2

The relationship of varying concentrations of dehy droascorbic acid to oxidized flavor in washed cream

Date	Control milk				Washed cream																Buffered or unbuffered
					210 mgm./l. dehydroascorbic acid*				105 mgm./l. dehydroascorbic acid*				42 mgm./l. dehydroascorbic acid*				21 mgm./l. dehydroascorbic acid*				
	p.p.m. copper				p.p.m. copper				p.p.m. copper				p.p.m. copper				p.p.m. copper				
	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5	
3/20/42	–†	1†	2†	2†	ox†	ox	ox	ox	ox	ox	ox	ox	ox	ox	ox	ox	–	ox	ox	ox	Buffered
3/21/42	–	1	1	1	–	–	–	–	–	ox	ox	ox	–	–	–	cx	–	–	–	–	"
3/23/42	–	1	2	2	ox	ox	ox	ox	ox	ox	ox	ox	ox	ox	ox	ox	–	ox	ox	ox	"
4/14/42	–	1	1	1	ox	ox	ox	ox	ox	ox	ox	ox	–	ox	ox	ox	–	ox	ox	ox	"
4/15/42	–	1	1	1	ox	ox	ox	ox	ox	ox	ox	ox	–	ox	ox	ox	–	ox	ox	ox	"

* Dehydroascorbic acid was prepared by oxidizing ascorbic acid with 0.1 *N* iodine solution and hence the solution added contained an amount of KI equivalent to the ascorbic acid added. † See table 1, footnote.

range. Accordingly, various concentrations of reduced ascorbic acid were tried in washed cream containing 0.1 per cent KI. The results of these experiments are shown in table 3. An examination of the data recorded in table 3 shows that in all cases containing 210 mg./l. ascorbic acid except two, the action of the potassium iodide was sufficient to protect the washed cream against the development of oxidized flavor. At concentrations of 40 to 100 mg. per liter of ascorbic acid, the oxidized flavor developed. This would appear to check with an earlier observation (8) that the strongest development of oxidized flavor occurred at these concentrations of ascorbic acid.

The protection of washed cream from the development of oxidized flavor by potassium iodide was unexpected. A partial explanation of this may be that the oxidation-reduction potential of hydrogen peroxide is much lower than that of potassium iodide. The standard method of determination of hydrogen peroxide is to add potassium iodide; the hydrogen peroxide liberates iodine which is then titrated with sodium thiosulfate. This, however, does not explain why potassium iodide did not protect with 40 to 100 mg. per liter of ascorbic acid. It seemed desirable to determine if potassium iodide has the same effect on milk. Accordingly, a number of experiments were planned using various concentrations of potassium iodide. Likewise it was thought advisable to determine if potassium chloride and sodium bromide had the same effect. In all these trials whole milk was used and various amounts of the salts in solution were added to give the desired percentage. The samples were stored and flavor determinations made in the same manner as the other experimental samples. The results are shown in table 4. These data reveal that 0.1 per cent KI in milk will protect against the development of metal-induced oxidized flavor. Lower concentrations give correspondingly lower protection. Concentrations of 0.1 per cent potassium chloride or 0.1 per cent sodium bromide give at most only a slight reduction in the intensity of the oxidized flavor developed.

The development of oxidized flavor in washed cream by ascorbic acid, glutathione, hydrogen peroxide, and dehydroascorbic acid, in the presence of copper, lends additional circumstantial evidence to the view that an enzyme is not involved in the development of oxidized flavor. To obtain further evidence on this point three samples of milk were obtained and treated in the same manner as the previous experimental samples except that the milk was pasteurized at a temperature not lower than 180° F. for 5 minutes prior to the washing of the cream. A sample of the raw milk was saved prior to pasteurization and served as a control sample. The results of this study are shown in table 5. Examination of the data reported in this table shows that a strong cooked flavor was found in all the samples of milk pasteurized at 180° F. after three days. However, in the washed cream a strong oxidized flavor was developed in all cases. Even when copper was not added, the presence of the ascorbic acid in the washed cream

TABLE 3

Relationship of varying concentrations of ascorbic acid to oxidized flavor in washed cream containing 0.1% KI

Date	Control milk				Washed cream																Buffered of unbuffered
					210 mgm./l. ascorbic acid				105 mgm./l. ascorbic acid				42 mgm./l. ascorbic acid				21 mgm./l. ascorbic acid				
	p.p.m. copper				p.p.m. copper				p.p.m. copper				p.p.m. copper				p.p.m. copper				
	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5	
2/10/42	–*	2*	3*	3*	–	–	–	–													Buffered
2/14/42	–	1	1	1	–	–	–	–													Buffered
2/17/42	–	1	1	2	–	–	–	–													Buffered
2/19/42	–	1	1	1	–	–	–	–													Unbuffered
2/21/42	–	1	2	2	–	–	–	–													"
2/24/42	–	1	2	2	–	–	–	–													"
2/28/42	–	1	1	2	.....	.....	.....	.....					ox	ox	ox	ox	ox	ox	ox	ox	Buffered
3/4/42	–	1	2	2	ox	ox	ox	ox	ox	ox	ox	ox	ox	ox	ox	ox	ox	ox	ox	ox	"
3/7/42	–	1	2	2	–	–	–	–	ox	ox	ox	ox	ox	ox	ox	ox	?	–	–	–	Unbuffered
3/9/42	–	1	2	2	–	–	–	–	ox	ox	ox	ox	ox	ox	ox	ox	–	–	–	–	"
3/10/42	–	1	1	1	–	–	–	–	ox	ox	ox	ox	ox	ox	–	–	ox	–	–	–	"
3/12/42	–	1	1	2	–	–	–	–	ox	ox	ox	ox	ox	ox	ox	ox	–	–	–	–	"
3/13/42	–	1	2	2	–	–	–	–	ox	ox	ox	ox	ox	ox	ox	–	.....	.....	.....	.....	"
3/19/42	–	1	2	2	ox	ox	ox	–	ox	ox	ox	–	ox	ox	ox	ox	ox	ox	ox	ox	Buffered

* See table 1, footnote.

TABLE 4

Relationship of varying concentrations of potassium iodide to oxidized flavor in milk

Date	Control milk				Milk + 0.1% KI				Milk + 0.05% KI				Milk + 0.01% KI				Milk + 0.1% KCl				Milk + 0.1% NaBr			
	p.p.m. copper				p.p.m. copper				p.p.m. copper				p.p.m. copper				p.p.m. copper				p.p.m. copper			
	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5
2/21/42	–*	1*	2*	2*	–	–	–	–																
2/23/42	–	1	2	2	–	–	–	–									–	?	1	2				
2/24/42	–	1	2	2	–	–	–	–													–	?	1	2
2/25/42	–	1	1	1	–	–	–	–					–	?	1	1					–	?	?	1
2/27/42	–	1	1	2	–	–	–	–					–	1	2	2					–	–	?	1
3/3/42	–	1	1	2	–	–	–	–	–	–	?	1	–	1	1	2	–	–	?	1	–	–	?	1
3/4/42	–	1	2	2	–	–	–	–	–	–	?	1	–	?	1	1	–	1	1	1				
3/14/42	–	1	1	1	–	–	–	–	–	–	–	1	–	?	1	1								

* See table 1, footnote.

TABLE 5

The relationship of high heat on milk to the development of oxidized flavor in washed milk

Date	Milk								Washed cream								Buffered or unbuffered
	Control				180° F.–5 min.				210 mgm./l. ascorbic acid				105 mgm./l. ascorbic acid				
	p.p.m. copper				p.p.m. copper				p.p.m. copper				p.p.m. copper				
	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5	
2/11/42	–*	2*	2*	3*	– H.H.†	– H.H.	– H.H.	– H.H.	ox	ox	ox	ox	.....	.....	.....	.....	Unbuffered
3/2/42	–	1	1	2	– H.H.	– H.H.	– H.H.	– H.H.	.....	.....	.....	.....	ox	ox	ox	ox	"
3/23/42	–	1	2	2	– H.H.	– H.H.	– H.H.	– H.H.	.....	.....	.....	.....	ox	ox	ox	ox	Buffered

* See table 1, footnote.
† H.H.—High heat or cooked flavor.

caused the development of the flavor in the limited number of cases studied. As enzymes are inactivated at high temperatures, these data show quite clearly that an oxidized flavor can be produced chemically without the aid of any enzyme and give further evidence that no enzyme is involved in the production of an oxidized flavor.

DISCUSSION OF RESULTS

The results of this experiment indicate that both ascorbic acid and dehydroascorbic acid are capable of producing an oxidized flavor in washed cream. In the case of dehydroascorbic acid it is necessary that the washed cream be buffered to control the pH since the flavor does not develop in strongly acid solutions. A review of the literature indicates that theoretically both the reduced ascorbic acid and the dehydroascorbic acid are capable of causing the development of oxidized flavor. This was found to occur under the conditions of this experiment. Likewise, it was found that the addition of 0.1 per cent potassium iodide to milk was sufficient to prevent the development of an oxidized flavor and that lesser amounts inhibited the flavor development to a lesser degree. Neither potassium chloride, nor sodium bromide were found to be as effective as potassium iodide. No complete explanation for this effect is evident at the present time.[1]

A study of high temperature pasteurization reveals that the sulfhydryls are contained in the plasma portion of the milk and are removed sufficiently when cream is washed so as to render the cream susceptible to the development of oxidized flavor with ascorbic acid. This would indicate that no enzyme is involved in the production of an oxidized flavor.

CONCLUSIONS

1. Washed cream fortified with ascorbic acid (210 mg. per liter) and contaminated with copper develops a strong oxidized flavor.

2. Washed cream fortified with dehydroascorbic acid (40 to 200 mg. per liter) and contaminated with copper develops a strong oxidized flavor when buffered.

3. When washed cream containing 210 mg. of ascorbic acid per liter is contaminated with copper the development of oxidized flavor is prevented in most cases by a 0.1 per cent concentration of potassium iodide.

4. When contaminated with copper, susceptible milk containing 0.1 per cent potassium iodide does not develop an oxidized flavor. Lesser concentrations of potassium iodide are ineffective in most cases.

5. Pasteurization of milk at 180° F. for 5 minutes prior to washing of the cream does not affect the susceptibility of the cream to oxidized flavor when contaminated with copper.

[1] Since submitting this article for publication, Mapson, (Biochem. Jour. 35: 1332–53, 1941) has shown that halides have a protective action on the oxidation of ascorbic acid in the order $I > Br > Cl$. He attributed the protective action to a copper halide complex ion.

ACKNOWLEDGMENT

The authors are indebted to Dr. H. K. Johnston, Department of Dairy Husbandry, for assistance in scoring the experimental samples of milk and washed cream; and to Dr. R. B. Dustman, Department of Agricultural Chemistry, for his assistance and constructive criticisms in the preparation of this paper.

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THE EFFECT OF OXYTOCIN ON MILK AND MILK FAT SECRETION[1]

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It was demonstrated previously that there was a marked decrease in the uptake of blood fat by the mammary gland immediately following milking, after which the fat utilization gradually increased (2). That the distension of the alveoli was necessary for the normal passage of fat into the gland was indicated by a number of experiments in which one half of the udder was milked out and the other half left distended with milk; arterio-venous blood samples for fat analysis were then drawn simultaneously from both sides and it was found that while the distended half used the normal amount of blood fat, the half milked out used little or no blood fat. The actual removal of the milk from the gland and not the "letting down" of milk was therefore responsible for the decrease in the utilization of blood fat.

From the above it appeared that by removing the milk from the gland at frequent intervals it should be possible to retard the passage of blood fat into the gland sufficiently to decrease the amount of fat secreted by the gland. Data are presented in this paper on the results of such a study, in which oxytocin was used to get complete ejection of milk at each milking. In addition, since oxytocin is being used as an aid in obtaining milk under certain experimental conditions, as in the case of the perfused gland (1), a study was made of the effect of oxytocin upon normal milk and fat production when injected at regular milking intervals.

EXPERIMENTAL

In the following studies obstetrical pituitrin was used to obtain complete ejection of milk from the gland and will be referred to as oxytocin.

The effect of oxytocin injections at regular milking intervals is shown in figure 1. The total milk and fat production is shown during a preliminary period of seven days, during a nine-day experimental period, and for seven days following the experimental period. During the nine-day experimental period the cow was milked normally twice each day. Immediately following each milking 10 I.U. of oxytocin were injected intravenously and the residual milk obtained. The milk drawn normally and the residual milk obtained with oxytocin were tested separately for fat. It will be noted that with the exception of the first day of oxytocin injection and the day after

Received for publication June 10, 1942.

[1] Presented before the American Dairy Science Assn., Eastern Division, September 14, 1941.

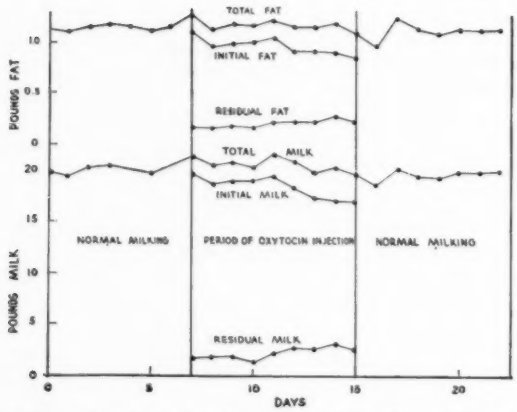

FIG. 1. The effect of oxytocin injections at regular milking intervals upon milk and milk fat production.

the last injection of oxytocin the total milk and fat production was not significantly affected by the oxytocin. The increased production of milk and fat on the first day was due entirely to the residual milk obtained with the first injection of oxytocin. The drop in production on the day following the cessation of oxytocin injection was due to the failure to obtain the residual milk on the first milking following the last injection of oxytocin. The amount of milk and fat actually secreted during the period, therefore, was not significantly changed by the use of oxytocin. During the nine-day period of oxytocin injection the normally drawn milk was lower in fat than during the control period, testing 5.24 per cent as compared to a normal of 5.72 per cent. However, the residual milk averaged 9.45 per cent so that the total milk obtained during the period of oxytocin injection did not differ significantly in fat per cent (5.69) from the milk obtained during the control

TABLE 1

Effect of milking at short intervals with the aid of oxytocin upon total milk and milk fat production
(Average of four cows)

	Milking interval	Length of period	Milk production	Fat production	Per cent of fat in milk
			lbs.	*lbs.*	
Preliminary period (normal milking).....	12 hours	24 hours (3-day average)	26.65	1.23	4.62
Experimental period (10 I.U. of oxytocin at each milking).....	2 hours	24 hours	26.22	1.06	4.04

period. During the period of oxytocin injection the first drawn or "initial" milk and fat decreased but was compensated for by an increase in the amount of residual milk and fat.

Four cows were milked at intervals of two hours for periods of from 30 to 40 hours. Ten I.U. of oxytocin were injected intravenously preceding each milking. The data are summarized in table 1. The first two milkings are not included in the averages as they were usually abnormally high in fat content due to the residual milk and did not represent the actual secretion during the period of oxytocin injections. The average production of the four cows during a 24-hour period in which the cows were milked every two hours is compared with the average daily production of these cows during a preliminary period of three days.

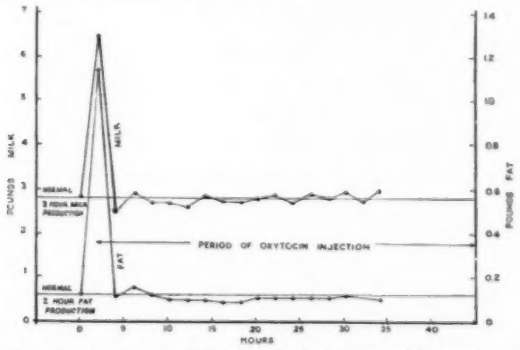

FIG. 2. Milk and milk fat production as affected by milking at two-hour intervals with the aid of oxytocin injections.

It will be observed in table 1 that while there was no marked reduction in the amount of milk produced during the experimental period, there was a decrease in the average fat production. This decrease occurred in each of the four cases under observation and averaged 13.8 per cent, whereas total milk production decreased only 1.6 per cent.

The detailed data on one of the animals, which is typical of the group, are presented diagrammatically in figure 2. The milk and fat production at two-hour intervals over a period of 34 hours is compared with the expected two-hour production on the basis of the previous three-day production. Following the first two injections of oxytocin the milk production at two-hour intervals varied less than seven per cent from the expected two-hour production in a period of 30 hours. The total milk production in the 30-hour period was identical with the rate of milk secretion during the three-day preliminary period. The fat production during the 30-hour period was 11.0 per cent less than the rate of fat production during the three-day preliminary period.

The various fat constants were determined on the butterfat obtained from the animal represented in figure 2 at four intervals during the 34-hour period, a composite being made of every four milkings in order to obtain sufficient fat for analysis. The Polenske and saponification values were not affected. The Reichert-Meissl value decreased during the first eight hours from 31.7 to 28.5 and remained at that level. The iodine value decreased from 30.1 to 21.6 during the first eight-hour period, declined to 21.3 during the second eight-hour period, and then increased to 27.8 and 28.5, respectively, during the next two eight-hour periods. The iodine values were also determined on the fat samples from two of the remaining three animals. In one case there was no appreciable change, while in the other there was a decline within 10 hours from 40.1 to 37.8. The reason for these variations is not apparent.

DISCUSSION

The finding that oxytocin does not inhibit milk secretion is not in agreement with the conclusion reached by Turner and Slaughter (3). However, the data presented by these workers are believed to indicate incomplete milking following the injection of oxytocin, due partially to the storage of residual milk during the subsequent milkings, rather than inhibition of milk secretion. A certain amount of residual milk is always present in the mammary gland following normal milking. When this milk is removed by the use of oxytocin the next milking is usually deficient by that amount unless oxytocin is again used, as some of the milk normally obtained at that time will be stored as residual milk.

Milking with the aid of oxytocin at intervals of two hours demonstrated that the rate of milk secretion is extremely constant and is not influenced by the removal of the milk from the gland. The decrease in milk fat secretion when the gland was milked out at short intervals can be explained on the basis of previous work (2), showing that the removal of milk from the gland decreases the uptake of blood fat. However, the decrease was not as large as might be expected on the basis of this work. Possibly the storage of fat within the glandular tissue was sufficient to supply most of the fat needed for normal fat secretion.

CONCLUSIONS

1. The injection of oxytocin at regular milking intervals did not affect the normal milk or fat secretion.

2. Milking at two-hour intervals with the aid of oxytocin did not affect total milk production but decreased milk fat secretion 11.0 per cent in 24 hours, probably by inhibiting the passage of blood fat into the glandular tissue.

3. The rate of milk secretion from hour to hour is comparatively constant and is not changed by the removal of milk from the gland at regular milking intervals or at shorter intervals of two hours.

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THE ERADICATION OF BOVINE TUBERCULOSIS*

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THE FIRST TWENTY-FIVE YEARS OF THE EFFORT

The attempt to eradicate tuberculosis from the cattle of this country took definite shape in 1917. It has touched most intimately the producers of milk since from their herds great numbers of cattle have been removed for slaughter. It has also been of interest to all those using milk as a raw material in dairy manufacturing since the healthfulness of the products is involved. It was thought that a review of the accomplishments of the quarter century and the indication of some of the conditions revealed in the work might be of interest to students of dairying.

On December 31, 1940, the Secretary of Agriculture made, in a radio address, the following statement: "Twenty-three years ago veterinarians believed as a result of research that tuberculosis of cattle could be stamped out in this country. This year *their* belief was *completely* vindicated. Tuberculin testing of cattle has now been completed in every county. The United States is *now practically free* of *bovine tuberculosis.*"

The Secretary of Agriculture was referring to the fact that in 1940 the entire country had reached the modified accredited status, *i.e.*, an area, usually a county, in which less than 0.5 per cent or five per 1000, of all animals tested react positively to a diagnostic dose of tuberculin.

The period of research to which the Secretary indirectly refers started in 1893–94, when the first tuberculin tests were made in this country. The research showed the value of tuberculin as a diagnostic agent for tuberculosis in the bovine and it also yielded the intradermal test without which the country-wide testing would have been impossible, since the original subcutaneous test would have been prohibitorily expensive. The period was also one of education of farmers as to the economic significance of the disease, of medical men as to the probable relation of bovine tuberculosis to tuberculosis in man, and of the general public to the same realization. Thus by 1917 the public was in sympathy with the use of public funds for the compensation of farmers whose cattle were found tuberculous. The farmers, having been convinced of the continued tax the disease placed on them, were, in large part, willing to submit their herds to examination and to bear a part of the loss incurred from the slaughter of reacting animals. An appropriate tool for diagnosis was available. Thus the stage was set for the great co-

Received for publication June 12, 1942.

* Published with the permission of the Director of the Wisconsin Agricultural Experiment Station, Madison, Wis.

operative endeavor by the Federal Government, represented by the Bureau of Animal Industry, and by the various state agencies responsible for livestock sanitation.

The effort is unquestionably the world's greatest in the field of disease eradication in the case of the domestic animals and possibly in all disease eradication. The plan was well conceived and the effort has been well sustained for a quarter of a century.

It seems proper to now review the work, to evaluate the results, and to view the problems of the future, since the task is not completed. The itali-

TABLE 1

Tuberculin testing under accredited-herd and area plans, 1917–41

Year ended June 30	Cattle tested	Reactors found and removed	
	Number	*Number*	*Per cent*
1917	20,101	645	3.2
1918	134,143	6,544	4.9
1919	329,878	13,528	4.1
1920	700,670	28,709	4.1
1921	1,366,358	53,768	3.9
1922	2,384,236	82,569	3.5
1923	3,460,849	113,844	3.3
1924	5,312,364	171,559	3.2
1925	7,000,028	214,491	3.1
1926	8,650,780	323,084	3.7
1927	9,700,176	285,361	2.9
1928	11,281,490	262,113	2.3
1929	11,683,720	206,764	1.8
1930	12,845,871	216,932	1.7
1931	13,782,273	203,778	1.5
1932	13,443,557	254,785	1.9
1933	13,073,894	255,096	2.0
1934	15,119,763	232,368	1.5
1935	25,237,532	376,623	1.5
1936	22,918,038	165,496	0.7
1937	13,750,308	94,104	0.7
1938	14,108,871	89,359	0.6
1939	11,186,805	60,338	0.5
1940	12,222,318	56,343	0.5
1941	13,229,499	40,702	0.3
Total	241,943,522	3,808,903	1.6

cized words in the quotation from the Secretary of Agriculture appear underlined in the official mimeographed release of the broadcast. It is certain that no one familiar with the work believes that the disease is completely eradicated, but rather believes that much effort and money must still be expended before the "mopping up" is completed. The extent and nature of the "mopping up" operations are questions of present significance.

The incidence of the disease revealed by the country-wide test was not as high as had been predicted from the preceding tests made in the areas in which, because of the length of time involved and the frequency of change in the herds by sale and purchase of animals, the disease had had much

greater opportunity to spread than in the usual herd or area. The findings emphasize the slowness with which bovine tuberculosis spread in the early years of the cattle industry of the country and indicate that a rapid increase from still unrevealed foci will not occur and that the apprehension of some regarding the recrudescence of the disease is not well founded. The record of Marathon County, Wisconsin, is evidence of this view. The dairy industry was well established therein by the beginning of the century, but when the first complete test of the 103,000 cattle was made in 1926, the incidence of reactors was but 0.53 per cent, a level not much above that of the entire country after 25 years of intensive effort.

The complete record of the eradication effort is presented in table 1, taken from the report of the Chief of the Bureau of Animal Industry for 1941. It should be remembered in connection with the data as to reactors that much of the testing in 1917–20 was in areas in which much testing had been done before 1917. It is estimated that nearly $300,000,000 of public funds have been spent in the effort, to which must be added the cost to the farmer. If one can judge from the experiences of England, in which the incidence of tuberculosis in cattle is over 40 per cent, the expenditure made is well worth while since, without the eradication program, the incidence in this country would have undoubtedly reached the English level at some future time. Indeed it had done so in certain areas in this country by 1920. The present generation has, in reality, purchased health and insurance against economic loss for future generations as regards bovine tuberculosis.

Certain states reached the stage characterized by the Secretary of Agriculture as practically free of bovine tuberculosis over a decade ago; others more recently. The data regarding these states are presented in Table 2, compiled from the monthly reports of the Bureau of Animal Industry. This record is that after the date of accreditation and should give some preview of the future record.[1] The record of certain states and counties shows that the time and effort demanded to reduce the incidence of reactors from a very high level to a low one is much less than to reduce the incidence from a low level to one falling much below the modified accredited level of 0.5 per cent.

The data for New York, accredited in 1935, and for California, accredited in 1940, are as follows:

NEW YORK

	Animals tested	*Reactors per 1000 tested*
1930	1,199,987	43.0
1934	1,938,579	80.0
1936	2,075,444	5.4
1940	1,363,237	4.9
1941	1,291,207	3.9

[1] The data for 1937 are not given since the record of that year was not available to the writer.

CALIFORNIA

	Animals tested	*Reactors per 1000 tested*
1935	961,121	86.0
1936	1,586,769	35.0
1940	1,571,313	6.8
1941	1,401,230	6.4

The same point is made evident by the record of two counties in Wisconsin.

KENOSHA COUNTY

	Year	*Reactors per 1000*
Original complete test	1927	398.0
Second complete test	1929	6.6
Third complete test	1932	7.7
Fourth complete test	1934	5.6
Fifth complete test	1937	1.0

MARATHON COUNTY

Original complete test	1926	5.3
Second complete test	1929	1.8
Third complete test	1932	1.9
Fourth complete test	1938	0.8

The states, the records of which are presented in table 2, represent both the north and the south, great dairy states such as Wisconsin and Michigan in which the herds are large and in which the cattle are stabled for a large part of the year, and other states in which the herds are small, the animals stabled only for short periods and probably the herds less subject to change by sale and purchase than the herds of the intensive dairy areas of the north. The sample used in table 2 would seem to be a fair one of the entire country and the record of the states included in table 2 one which will later be duplicated by other states.

In 1917 the dominant idea, indeed the only idea, was that the sensitization of the bovine to tuberculin was always occasioned by the bovine tubercle bacillus, the organism that can and does cause progressive and fatal tuberculosis in the bovine. It was, therefore, concluded that every reacting bovine is a focus from which tubercle bacilli are passing or may, at some future time, pass to the bovine associates of the reacting animal, to swine and to humans. The removal of every reacting animal for slaughter was, therefore, fully justified, and as is shown in table 1, 3,808,903 animals were removed and slaughtered in the period 1917–1941 inclusive, a number equal to about 7 per cent of the total number of cattle in the country at any one time.

This supposition had another implication; namely, a reasonable hope that, within a period of years, all sources of infection would be removed, the foci from which the organism could pass to still healthy cattle would no longer exist, and no further reactors would be found. The end of the road

TABLE 2

The incidence of animals reacting to tuberculin expressed in units per 1,000 units tested

	1929	1930	1931	1932	1933	1934	1935	1936	1938	1939	1940	1941
North Carolina	1.9	1.2	1.6	2.4	1.5	3.2	1.8	0.3	0.1	1.2	0.03	0.22
Maine	4.4	4.0	3.3	4.0	2.7	1.4	2.6	1.3	1.3	1.0	0.9	0.63
Michigan	....	4.6	3.7	1.7	1.3	2.2	1.6	1.3	0.9	1.8	2.3	2.3
Indiana	....	....	5.2	4.8	5.8	5.4	3.6	3.4	3.0	2.5	2.0	1.5
Kentucky	....	....	....	....	....	2.9	1.5	1.3	0.6	2.7	3.0	1.8
West Virginia	....	....	....	....	....	1.4	1.6	1.1	2.8	1.3	2.3	1.4
Washington	....	....	....	....	....	7.0	5.6	4.1	5.4	2.0	1.5	2.0
Wisconsin	....	3.8	3.2	5.0	6.1	2.6	1.9	2.1	1.9	2.9	1.8	1.7

would have been reached. The only obstacle in the way seemed to be the tuberculous animal, the skin of which was not sensitized to tuberculin by the growth of the bovine tubercle bacillus in its tissues, or again the animal the tissues of which had been made insensitive to tuberculin, a condition possibly obtaining in far advanced cases of the disease. The latter type should pass from the herd by death or sale as a discard; the first type might continue in the herd, but not for a period much longer than a cow generation, which is, in most dairy areas, not much over six years. The ease with which the incidence of bovine tuberculosis was reduced from a very high level to a low one is evidence that this type of animal cannot be an important factor in the persistence of reactors, the phenomenon illustrated by the data of table 2.

Other evidence of the hope that a definite stopping place would be reached was the attitude toward the reacting animal that showed no evidence of tuberculosis on post-mortem examination. The expression first used to refer to such animals was "no-lesion"; later changed to "no-visible-lesion." The latter seemed proper since it was thought that all such were tuberculous, but that for one reason or another the lesions were not detected. Evidence slowly accumulated to show that the matter was not so simple as had been earlier thought. In those areas in which the disease had reached a high level of incidence, one would expect to find only few of the no-visible-lesion animals among the reactors, while in the areas of low incidence they would form a larger part of the reacting group. In the repeated tests of the cattle of any area, the foci from which bovine tubercle bacilli could spread were being removed. Indeed the evidence points to very complete removal of such foci. This would tend toward the elimination of the type of cases thought earlier to be responsible for the no-visible-lesions.

If, however, one admits that a bovine may be sensitized to tuberculin by some relative of the bovine tubercle bacillus not able to p· oduce a progressive type of disease, the record presented in table 2 seems to make sense, but does not make sense on the basis of the prevalent ideas of 1917. The incidence of the reactor in Wisconsin in 1941 is not much less than in 1935; in Michigan the value for 1941 is greater than that for 1932. The records of the other states seem to offer more hope of reaching the final goal of no reactors, no more testing, and no more expense to government or to individuals. Now and then the record of a state indicates the near approach to the desired goal of no reactors. Thus, in Arkansas in the 3-year period, September 1938 to October 1941, no reactors were found in approximately 55,000 cattle. In October 1941 one reactor was found, and in November two. In other states a greater number of animals have been tested without a reactor than in Arkansas, but in most cases the goal seems from the present record unattainable, at least if present methods continue to be used. The desired goal is freedom from tuberculosis caused by the bovine tubercle bacillus, not what was once thought to be the same goal, no reactors to tuberculin.

All now agree that the avian tubercle bacillus can and does sensitize bovines to the usual tuberculin used in this country, made with the human tubercle bacillus. It is evident that if the goal of no reactors is to be reached, tuberculosis in farm poultry must be eradicated. The report of the Bureau of Animal Industry for 1941 states that avian tuberculosis was found on 22 per cent of 11,454 farms inspected in the midwestern and north central states. The disease in poultry can be quickly and cheaply eradicated by proper management of the flocks.

In most areas some of the reacting cattle show no internal lesions of tuberculosis, but do show lesions on the skin of the legs in which acid-fast bacilli can be found on microscopic examination. Earlier it was thought these were true bovine tubercle bacilli, but the inability to cultivate them on artificial media in numerous trials extending from 1930 to the present indicates that the earlier idea is no longer tenable. There is no reason to believe that this unknown organism is able to cause serious trouble in the infected animal, or is of significance in other relations. The ability of the organism to sensitize the animal to tuberculin and its resistance to cultivation indicate the possibility that other similar organisms may occur in other regions of the body.

The human tubercle bacillus has been emphasized as a possible sensitizing agent for cattle. Observations made both in the field and under experimental conditions in Finland indicate that this agent is a disturbing factor in the attempted eradication of bovine tuberculosis in that country. There the final stages in the effort seem to be quite similar to those appearing in certain areas of this country. The conditions existing in this country are so different from those of Finland that even though the human tubercle bacillus may be significant in relation to the sensitization of cattle in Finland, it is not likely to be of like importance here.

The acid-fast organism causing Johne's disease in cattle and in sheep may also prove to be a confusing factor of significance as may other members of the group of bacteria of which the tubercle bacilli are members. Such organisms are widely distributed in nature, especially in soil, on plants and in and on the animal body. Their constant occurrence in the fatty secretions of man is a well-known fact.

The sensitization of man by other than the human and bovine tubercle bacilli, the forms pathogenic for man, is being recognized by the physician using the tuberculin test. He views the test as a sieve by which he can divide any group into two parts: the nonreactors which he can dismiss and the reactors which must be examined by x-ray and by other methods in order to determine whether the reacting individual is actually tuberculous at the moment, or that the present sensitization is due to a past infection with human or bovine tubercle bacilli that has been overcome or to some other and possibly harmless organism.

The present record of those states that reached the modified accredited status some years ago indicates that the record of the future will be much the same and that there is little hope of reaching the goal, *i.e.*, the time when no reactors would be found, which in 1917 seemed possible. It seems that some reactors will continue to appear and that testing must be continued.

The question of continued slaughter of cattle is more difficult to answer. This should and must continue so long as seems necessary to safeguard the position won in the 25-year battle. The answer as to what is necessary to safeguard the present position cannot be given from present knowledge. There is no question but that some part of the cattle now removed for slaughter could be left in the herds without harm to the herd since the sensitization is due to the avian or to the human tubercle bacillus, or to some not yet cultivated member of the group. It is to be remembered that no one of these organisms is capable of causing death of the bovine or of reducing its productive capacity or is of importance as regards the healthfulness of milk, since the organisms would not be excreted, even if present in the tissues.

The fact that at least one of the agents sensitizing cattle to tuberculin has, to the present, resisted cultivation indicates that there may be others of like nature in the picture. The possibility also exists that the pathology of the infection may be diffuse rather than definite, thus making a microscopic examination for the organisms difficult. If the skin-lesion infection was diffuse rather than definite, it might not have been detected. These possibilities indicate the need of expanding the research in this field by other methods than have been commonly used; namely, gross and microscopic pathology and cultural methods either in vivo with experimental animals or in vitro. Feldman has recently reported his findings on 101 reacting animals removed from 99 herds. They indicate that 77.2 per cent were not infected with the bovine tubercle bacillus. Even those that had been classed as showing lesions on post-mortem examination were in 12 out of 44 cases infected with a sensitizing agent other than any of the types of tubercle bacilli.

The reacting animal itself must be studied as to its future conduct in the environment in which it was discovered or in a changed environment. It must be kept to write its own record. Will it continue to react if removed to another environment, thus breaking the connection between the animal and the source of the sensitizing agent, which must be constantly supplied if the sensitization is necessarily to persist? Thus, cattle can apparently be kept sensitive to the avian tubercle bacillus only by constant association with tuberculous birds, and it has even been shown that some humans that have recovered from tuberculosis lose the sensitivity to tuberculin when they are not constantly associated with other tuberculous individuals, and retain it when the association is continued. The greatest information would be gained by allowing all reacting animals to remain in the herds when a

definite history of infection with the bovine type is not established. Such trials must be on an experimental basis in certain selected areas. The plan involves research on the farm and of course must provide for the payment of all costs and damages suffered by the owner of the herd. The trials should be sufficiently numerous and comprehensive to indicate the relative safety of using the information thus secured to contradict that supplied by the tuberculin test in much the same manner as the radiologist uses the information gained by x-ray or fluoroscope to confirm or to contradict the tuberculin test in man. The reports as to the number of cattle tested each month in the different states, and as to the number of instances in which "no reactors found" supply data indicating the rate of progress toward the goal of "no reactors."

	Trials	*Trials in which no reactors found*	
1938	601	78	13 per cent
1939	591	80	13 " "
1940	594	92	15 " "
1941	593	94	15 " "

"Thus, out of 601 trials no reactors were found in 78 instances in 1938. The data indicate the slowness of the progress toward the goal of no reactors. Indeed, whatever progress is indicated by these data may be offset by the reduction in number of animals tested in any area in any month. Reactors are more likely to be found if many animals are tested than if few are tested. There has been a marked decrease in the frequency of testing all the cattle in certain areas. Thus, in Wisconsin there was a six-year interval between the third complete test and the fourth in the case of Marathon County and over four years between the fourth complete test in Kenosha County and the fifth. There seems to be no question concerning the wisdom of gradually lengthening the interval between examinations of all cattle in any area.

The hesitancy of those in control of programs related to human and animal sanitation toward the modification of these programs is commendable, and it is probably too much to expect that modification will be suggested by those actively engaged in the effort. Every such program at some time requires adjustment to the new conditions revealed by the program in action. Thus the program related to yellow fever is not now that of a decade ago because the old program in action revealed that yellow fever is not a disease of man alone transmitted by a specific mosquito alone, but that the causal organism has other foci than man and other vectors than the specific mosquito. The old must be supplemented or modified by the new and unless this is done in the field of bovine tuberculosis, it seems that the next decade will see the possibly unnecessary expenditure of public money and the possibly unnecessary slaughter of cattle. The situation demands, as Feldman[2] suggests, the re-examination of the question as to the possibility of other agents than tubercle bacilli sensitizing cattle to tuberculin.

[2] Amer. Jour. Vet. Res., 3: 3–9, January, 1942.

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VOLUME XXV DECEMBER, 1942 NUMBER 12

JOURNAL OF DAIRY SCIENCE

Published by the
AMERICAN DAIRY SCIENCE ASSOCIATION
R. B. STOLTZ, Sec.-Treas.
Ohio State University, Columbus, Ohio

ABSTRACTS OF LITERATURE

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Columbus, Ohio

MILK AND MILK PRODUCTS

Published in cooperation with
INTERNATIONAL ASSOCIATION OF ICE CREAM MANUFACTURERS
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THE SCIENCE PRESS PRINTING COMPANY LANCASTER, PA.

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ABSTRACTS OF LITERATURE

BOOK REVIEWS

727. Pumps, Types, Selection, Installation, Operation, and Maintenance. FRANK A. KRISTAL, Mech. Eng., Weinman Pump Mfr. Co., AND F. A. ANNETT, Assoc. Ed., "Power." McGraw-Hill Book Co., Inc., New York. 1940.

These authors have attempted to cover the subject of pumps from the standpoint of practicality, that is, operational application, rather than the most common treatment encountered, that of design problems. Therefore, after a description of the many industrial types of pumps, including operating principles and construction materials, comprising the first ten chapters, the authors take up in the succeeding eight chapters the subjects: Methods of Priming Pumps; Pump Operations; Drives for Pumps; Pump Selection, Installation, and Operation; Centrifugal-pump Troubles and Remedies; New Pumps for Old. This book contains much information which should prove valuable to the dairy plant operator and to the dairy equipment sales engineer. Very well illustrated. L.M.D.

728. Industrial Chemistry of Colloidal and Amorphous Materials. WARREN K. LEWIS, Mass. Inst. Technol., LOMBARD SQUIRES, DuPont de Nemours Co., AND GEOFFREY BROUGHTON, Eastman Kodak Co. The Macmillan Co., N. Y. 1942.

This book prepared as a text on the industrial chemistry of colloidal and amorphous materials is of particular interest to those in the dairy industry engaged either in research along such lines or in the technical applications of research findings because of its clear-cut treatment of the basic physical chemistry phenomena involved and understanding of which is necessary in the interpretation of industrial problems arising in technical applications. Thus the first half of the text is devoted to such subjects as: structure of liquids, viscosity, surface tension, surface tension and orientation, adsorption, suspensoids, amorphous solids, emulsoids, the electrochemical behavior of colloids, gelation, emulsions and foams, and crystalline and amorphous states. The balance of the subject matter is devoted to industrial aspects largely outside of the dairy field. L.M.D.

BACTERIOLOGY

729. An Emergency Method for Estimating Bacterial Populations (A Preliminary Report). RAY HASSON, Griffin-Hasson Lab., Los Angeles, Calif. Jour. Milk Technol., *5*, No. 4: 243. July-Aug., 1942.

Ten milliliters of standard agar media (2% agar) is sterilized in two-

ounce square-bottomed flint glass bottles fitted with molded bakelite caps. While agar is still melted, the bottles are laid on their sides until media has solidified. The agar is then flooded with the samples of milk to be examined, drained and then incubated. Sterile skimmilk is recommended for making dilutions when necessary.

The quantitative results obtained may be correlated with an actual plate count and a factor obtained which may be used for future determinations under like conditions.

No mention is made of the amount of milk used for flooding the agar slab nor the length of time it should be drained. L.H.B.

730. **Detection of Thermoduric and Thermophilic Bacteria.** A. MOLDAVAN, Guaranteed Pure Milk Co., Ltd., Montreal, Canada. Canad. Dairy and Ice Cream Jour., *21*, No. 4: 36. 1942.

In the simplified technique described in this paper the three usual operations, test-tube pasteurization, diluting or looping, and agar-tubing may be carried on simultaneously. When more specific information is desired, four sets of separate tests may be carried on simultaneously to differentiate true thermoduries from milk-protected thermoduric and thermophilic bacteria and at the same time establish the rate of survival of such types under laboratory conditions. These tests are raw milk count, laboratory-pasteurized milk count, laboratory-pasteurized 0.001% dilute milk count, and laboratory-pasteurized milk count incubation at 60° C. The calibration and use of a special platinum loop is described. Bacteria surviving pasteurization temperatures in a 0.001 ml. milk dilution in distilled water may be termed true thermoduric and often are similar in types to those found in poorly sterilized cans. O.F.G.

731. **Biennial Reviews of the Progress of Dairy Science. Section B: Bacteriology and Mycology Applied to Butter.** ANONYMOUS. Jour. Dairy Res., *12*, 350–390. 1941.

Recent pertinent literature is reviewed under the headings: (1) Milk control, technique (90 references); (2) Lactic acid and allied fermentations, (a) bacterial metabolism, (b) lactic acid bacteria, (c) starters, (d) cheese, (e) butter (228 references); (3) Pasteurization and other processes, (a) pasteurization, (b) other processes, (c) canned and dried milk (65 references); (4) Detergents and disinfectants (24 references). S.T.C.

BUTTER

732. **Reducing the Loss of Fat During Churning.** G. H. WILSTER, R. P. ROBICHAUX, R. E. STOUT, AND R. W. STEIN. Oregon State College Station Bul. 397. July, 1941.

This study was based upon observed butterfat losses in buttermilk in

Oregon creameries and the influences of specified recommended practices upon these losses. The percentage of fat in the buttermilk was determined by the Babcock-butyl-alcohol-sulphuric-acid method of McKay and Mitchell and the results agreed well with those secured by the Mojonnier method. The results were expressed as the per cent of the total churned fat which was lost in the buttermilk.

In the college creamery and well-operated commercial creameries from 1.0 to 1.25% of the fat in the cream was lost in the buttermilk. These losses are considered to be minimum values. When losses of fat were great they could be reduced by several simple practices. The cream should test 36 to 40% fat. Low test cream gives more buttermilk that tests higher in fat. The water rinse should not be added directly to the churn but should be separated and this cream used for churning. The pasteurized cream should be cooled to 40°–45° F. and held at least three hours before churning, preferably overnight. The churn should not be overloaded and the cream churned at a temperature to require at least 45 minutes to churn. Some plants were found that were losing a minimum of fat while one creamery saved $20 per day through more exhaustive churning by following these recommendations.

A.C.D.

733. **The Rheology of Butter. I. Methods of Measuring the Hardness of Butter.** R. M. Dolby, Dairy Res. Inst. (N.Z.). (D.S.I.R.) Palmerston North, New Zealand. Jour. Dairy Res., *12*, 329–336. 1941.

An apparatus is described in which the resistance of butter to cutting by a wire is measured. The results are expressed as the load in grams required to produce a given rate of cutting. The results were found to be in general agreement with, but much more reproducible than those secured using the Scott Blair apparatus in which hardness is measured by compression of a cylindrical sample.

S.T.C.

734. **The Rheology of Butter. II. The Relation between the Rate of Shear and Shearing Stress. The Effect of Temperature and of Reworking on Hardness and on Structural Viscosity.** R. M. Dolby. Dairy Res. Inst. (N.Z.). (D.S.I.R.) Palmerston North, New Zealand. Jour. Dairy Res., *12*, 337–343. 1941.

It is shown that the rate of shear of butter when cut by a wire increases as a power of the load applied to the wire. The values determined for this power were high (index $n = 8$ to 15, average 11 at 12.5° C. (54.5° F.) and show a high structural viscosity in the butter. Although there was no indication that butter exhibits a true yield value the (rate of shear)/(load) curve is such that a practical yield value can be found. The practical yield

value (defined as the minimum load required to produce a measurable rate of flow) was used as a measure of the hardness of butter. The hardness of a number of samples of butter was determined over the range 6–18° C. (42.8°–64.4° F.). The hardness of the butters showed a steady fall with increase in temperature but the rate of decrease was not the same for all the samples so that their order of hardness changed as the temperature was increased. Reworking was shown to decrease the hardness to about one-fourth of the original value. There was a slow recovery on standing.

S.T.C.

735. **The Rheology of Butter. III. The Effect of Variation in Butter-making Conditions on the Hardness of the Butter.** R. M. DOLBY. Dairy Res. Inst. (N.A.). (D.S.I.R.)Palmerston North, New Zealand. Jour. Dairy Res., *12*, 344–349. 1941.

Slowly cooled cream was found invariably to yield butter which was softer than that churned from rapidly cooled cream. The use of low temperature wash water tended to reduce the hardness of the butter but the effect was small. The other factors studied had no consistent effect on the hardness of the butter. These were the type of pasteurizer, temperature during holding and during churning and amount of working. S.T.C.

736. **Observations on Bacterial Discoloration of Butter.** A. H. WHITE, Dairy Specialist, Sci. Serv., Ottawa, Canada, Canad. Dairy and Ice Cream Jour., *21*, No. 7: 19. 1942.

Discoloration of butter which contained from 1.5 to 2.0% salt has been found due to *Pseudomonas nigrifaciens*, although on laboratory media the organism does not produce pigment with 5.0% salt or more. The defect was also reported for the first time on the surfaces of butter boxes which had been held at temperatures of 33° to 40° F. Case histories and experimental trials indicated that the discoloration had developed because of extraneous moisture at the surface of butter. The organism has been isolated from floors, drains, and a butter printing table in plants which have experienced trouble. The organism is easily destroyed by pasteurization at 170° F. for one minute. O.F.G.

737. **What War Has Done to Fats and Oil Supplies.** G. W. MCBRIDE. Food Indus., *14*, No. 2: 57–59. 1942.

Warfare in the Pacific has reduced our imports of fats and oils. Primary fats and oils have not been increased in production domestically to meet our needs during the past 30 years.

In 1942, however, a 35% increase in lard production over the 1936–40 average is anticipated. Flax seed production in 1942 will approach record production; and Canadian and Argentine surpluses are available.

The production and trade in oils indirectly affect milk fat economics. Regardless of the balance maintained between animal and vegetable fats, specific fats will be subject to shortages although there is, by a price-fixing plan, a tendency to reduce shortages in the fat and oil supplies. J.C.M.

CHEESE

738. How to Use Waste Rind in Processed Cheese. SIMON BRICKNER, Consulting Chemist, Brooklyn, N. Y. Food Indus., *14*, No. 3: 47. 1942.

Natural Swiss cheese is covered with a rind a quarter of an inch thick. Natural Swiss cheese is used in making processed Swiss cheese and part of a blend of American cheese. Before cooking the rind has usually been thrown out but it has been found to contain 35% fat and 20% moisture. This is ideal for processing. In a special kind of hammer mill it can be ground to a fine powder and up to one third of a batch replaced by it without bad results. A higher overrun is possible because of its low moisture content.

This process used commercially for past one and a half years has been very gratifying. It may lower cost of production and increase the amount of processed cheese. J.C.M.

739. A Simplification of the Scott Blair—Coppen Test for the Pitching Consistency of Cheese Curd. G. W. SCOTT BLAIR AND F. M. SCOTT BLAIR. Natl. Inst. Res. in Dairying, Univ. Reading, Eng. Jour. Dairy Res., *12*, 322–328. 1941.

The simplification of the Scott-Blair Coppen test for the pitching consistency of cheese curd (reviewed in JOUR. DAIRY SCI., *24*, A26, 1941) consists in increasing the time for each stage in the process. The test previously carried out in 50 seconds, each process being carefully timed to the nearest second, can be as effectively done more slowly if the same proportionate times are used for each stage. The timing need not be highly accurate.

S.T.C.

740. Organisms Causing Rusty Spots in Cheddar Cheese. C. S. PEDERSON AND R. S. BREED, N. Y. State Agr. Expt. Sta., Geneva, N. Y. Canad. Dairy and Ice Cream Jour., *21*, No. 8: 19. 1942.

Rusty spot outbreaks in cheddar cheese have been reported from time to time and according to the results of the study reported in this article are caused by chromogenic varieties of *Lactobacillus plantarum* var. *rudensis* and *Lactobacillus brevis* var. *rudensis*. The first organism produces a trace of carbon dioxide in its production of lactic acid from glucose and other carbohydrates, whereas the second produces, in addition to lactic acid, car-

bon dioxide, alcohol, and volatile acids from dextrose and mannitol from levulose. Non-chromogenic strains of the two organisms are commonly present during the ripening of all cheddar and similar types of cheese.

O.F.G.

741. Some Other Activities of the Dairy Products Board. J. F. SINGLETON, Chairman, Dairy Products Board. Canad. Dairy and Ice Cream Jour., *21*, No. 4: 19. 1942.

The Board has been active in promoting the saving of domestic veals suitable for the manufacture of rennet, has purchased 100,000 veals from New Zealand, and has distributed these at cost to the two Canadian manufacturers of rennet. Provisions are being made to substitute pepsin for rennet in the manufacture of cheese if it becomes necessary even though pepsin does not make as satisfactory cheese as rennet. The Board also has been active in securing the release of black iron, tinned iron, and block tin for the construction of cheese vats and milk cans. There has been a shortage of properly seasoned cheese boxes.

Milk production during 1941 exceeded that of 1940 by about a billion pounds or about 6%. An increase of 500 million pounds for 1942 over 1941 seems desirable to take care of the increased domestic demands and to provide 125 million pounds of cheese for the United Kingdom. O.F.G.

CHEMISTRY

742. Effect of Light on Riboflavin Solutions. Effect of Sunlight on Reduced and Unreduced Solutions. C. M. O'MALLEY AND C. W. SIEVERT. Amer. Dry Milk Inst., Inc., Chicago, Ill. Jour. Indus. and Engin. Chem., Indus. Ed., *34*, No. 9: 1117. Sept., 1942.

Two extracts of skim milk solids and one solution of synthetic riboflavin were prepared for 30 minute periods of exposure to sunlight. To a portion of each extract, sodium hydrosulfite was added to maintain the riboflavin in a reduced state during exposure. About 90% of the riboflavin in the unreduced solutions was destroyed by the exposure but only a small portion of the reduced riboflavin was lost. Reduction of riboflavin with an unstable reducing agent did not protect the solution when heat was applied.

B.H.W.

743. Measuring the Concentration of Dissolved Oxygen in Dairy Products. A Voltammetric Method. G. H. HARTMAN AND O. F. GARRETT, N. J. Agr. Expt. Sta., New Brunswick. Jour. Indus. and Engin. Chem., Analyt. Ed., *14*, No. 8: 641. Aug., 1942.

The method of measuring dissolved oxygen by the dropping mercury electrode has been applied to milk. The method determines the concentra-

tion of dissolved oxygen in the aqueous phase of a milk product and the quantity of dissolved oxygen is calculated after considering the solids content of the product. The slight variations in the solids content of normal milks did not introduce significant errors. A linear relationship was observed between the concentration of dissolved oxygen in milk and the magnitude of the galvanometer deflections. The concentration of oxygen in milk may be determined at a potential ranging from 0.8 to 1.2 volts. The method gave reliable results when used in the determination of dissolved oxygen in a number of commercial milk samples. B.H.W.

744. **Oxidative Destruction of Vitamin D.** J. C. Fritz, J. L. Halpin, J. H. Hooper, and E. H. Kramke. The Borden Co., Elgin, Ill. Jour. Indus. and Engin. Chem., Indus. Ed., *34*, No. 8: 979. Aug., 1942.

A substantial loss in potency may occur in the vitamin D added to dry carriers, such as dry whey, which are used in mixed feeds. Some feeds show marked loss of vitamin D during three-month storage at room temperature; others show no loss during six-month storage. All the sources of vitamin D tested were susceptible to destruction. There was little evidence of a lag or induction period in this destruction. Oxidation was shown to be the cause of vitamin D loss and any condition which promoted oxidation such as increased surface area or the presence of fat which became rancid during the storage period accelerated the destruction. Various methods of stabilization were tested. Protection resulted from elimination of air contact and storage at low temperatures. Different coating agents such as calcium stearate were found to effectively retard oxidation when they were used to form a protective coating around the feed particles. If the impregnated particles were completely covered, the nature of the insulation had little effect on the protection afforded. Antioxidants such as oat flour were helpful in retarding oxidation. B.H.W.

745. **Extraction and Assay of Nicotinic Acid from Animal and Plant Tissues. Comparison of Methods.** Veron H. Cheldelin and Robert R. Williams, 297 Summit Ave., Summit, N. J. Jour. Indus. and Engin. Chem., Analyt. Ed., *14*, No. 8: 671. Aug., 1942.

Various aspects of the problem of determining nicotinic acid in a number of natural substances have been studied. Seven enzymes were employed for digestion, takadiastase and papain appearing to liberate nicotinic acid completely from a variety of materials. Enzymatic digestion or acid or alkaline extraction gave the same values for nicotinic acid content of meats and milk while with cereals the acid and alkaline treatments gave higher values. B.H.W.

746. Growth Stimulants in the Microbiological Assay for Riboflavin and Pantothenic Acid. J. C. BAUERNFEIND, A. L. SOTIER, AND C. S. BORUFF, Hiram Walker and Sons, Inc., Peoria, Ill. Jour. Indus. and Engin. Chem., Analyt. Ed., *14*, No. 8: 666. Aug., 1942.

Stimulants for the growth of *L. casei* in the microbiological assay for riboflavin and pantothenic acid when suboptimum amounts of the vitamin are present in the assay medium have been shown to exist in some foodstuffs including dried skim milk. The presence of the stimulants has been found responsible for discrepancy in results in assay of riboflavin and pantothenic acid. Stimulatory action of aqueous extracted residues, photolyzed aqueous suspensions of foodstuffs, extracted residues of distillers' dried solubles, and a number of known compounds is cited. The stimulatory action may be avoided by use of clarified aqueous extracts; preliminary lipoid solvent extraction; inclusion of photolyzed extracts of the product in the riboflavin assay medium, or inclusion of acid or alkaline-treated extract in the pantothenic acid assay medium. B.H.W.

747. Ethyl Alcohol from Lactose in Whey. H. H. BROWNE, Bur. Dairy Indus., U.S.D.A., Washington. Canad. Dairy and Ice Cream Jour., *21*, No. 8: 23. 1942.

Fermentation of whey lactose to ethyl alcohol by three organisms, *Torula cremoris*, *Torulopsis sphaerica*, and *Torula lactosa*, is described. The percentage of the theoretical yield varied from 25.3 to 83.6 with the different cultures used. The economic practicability of fermenting the lactose in whey will depend upon local operating conditions. The sulfite liquors used in the manufacture of ethyl alcohol do not average over three per cent of fermentable sugars while whey contains not less than 4.5% of lactose. While the cost of whey assembled for fermentation is uncertain, it is probable that its advantage over molasses would be in the lower cost of raw materials. Its disadvantage is in the higher steam cost which is about three times that of distilling a molasses mash. O.F.G.

CONCENTRATED AND DRY MILK: BY-PRODUCTS

748. Preventing Off-Flavors in Dried Whole Milk. E. L. JACK AND J. L. HENDERSON. Food Indus., *14*, No. 3: 50–52. 1942.

Dried whole milk has been increasing in its use but evaporated still exceeds it. One reason for its scant production is its low keeping quality. Within 60–90 days after production it develops a tallowy taste due to oxidation of fat. Experiments show that dry whole milk, made by the atmospheric drum process, keeps better. Low temperatures aid also.

This article goes into detail on the Buflovak Experimental Dryer, gives data, results, summary and references to dried whole milk studies. J.C.M.

749. Milk Solids and Bread Enrichment. H. H. MITCHELL, T. S. HAMILTON, AND J. B. SHIELDS, Univ. Ill., Urbana. Natl. Butter and Cheese Jour., *33*, No. 9: 12. Sept., 1942.

In the manufacture of patent white flour some of the calcium, phosphorus, iron, thiamin, niacin and riboflavin are lost. When these substances are added to the flour for bread making, the flour and the bread are said to be "enriched." Feeding tests with rats show that 6% milk solids used in white bread markedly improved its nutritive value. Whole wheat bread and enriched bread with 6% milk solids excelled enriched bread with added calcium and riboflavin. Smaller amounts of whole wheat bread with 6% milk solids were required to produce definite gains in weight as compared with whole wheat bread and white bread with 6% milk solids. The presence of milk solids improved bone formation; increased riboflavin in the body, as did whole wheat bread, and improved retention of niacin; but does not improve concentration of blood hemoglobin over enriched bread or whole wheat bread; and does not maintain the thiamin content as do these two latter types of bread. W.V.P.

DISEASE

750. Homogenized Sulfanilamide-in-Oil Intramammary Injections in Bovine Mastitis. J. C. KAKAVAS, C. C. PALMER, JAMES R. HAY, AND EDWARD S. BIDDLE, Newark, Delaware. Amer. Jour. Vet. Res., *3*, No. 8: 274. July, 1942.

In vitro studies with several strains of *Str. agalactiae* revealed that a concentration of 20 mg. per cent of sulfanilamide, which is near the limit of tolerance, did not destroy the organisms in 70 hours at 37° C. (98.6° F.), but effectively destroyed them at 40.5° C. (104.9° F.). Different strains resisted from 0.1 to 1.0% concentration at 37° C.

A 38% suspension of homogenized sulfanilamide in a light, liquid petrolatum was injected into individual quarters in 40 cc. doses at 24-hour intervals until four injections had been given. Larger quarters were given 80-cc. initial doses. Repeated treatment was administered when indicated. Of 100 cows injected with *Str. agalactiae* and three with *Str. uberis* in a total of 265 quarters, 89.3% of the cows and 94.7% of the quarters were freed of infection including all infected with *Str. uberis*.

Sulfanilamide concentration in the milk was high, but remained low in the blood taken from the subcutaneous abdominal vein. Treatment was carried out at any time and there were no contraindications and no reactions to the treatment.

Sulfanilamide in oil in 80-cc. doses was effective against pathogenic strains of *Staph. aureus* unless udder necrosis had developed. Sulfathiazole in oil was also effective in smaller doses, but caused formation of casein curd which was difficult to remove from the udder. S.A.F.

751. The Relation of Flies (*Musca Domestica Linnaeus*) to the Transmission of Bovine Mastitis. H. E. EWING, JR., Newark, Del. Amer. Jour. Vet. Res., *3*, No. 8: 295. July, 1942.

With the methods used, it was found that all *Musca domestica* Linn. examined carried *Str. agalactiae* on their external surface for two days, 20% for three days, 6% for seven days and none for eight days. Some flies carried the organism internally for 10 but not for 14 days. Of seventeen cows carrying either *Str. agalactiae* or pathogenic staphylococcic infection, 16 showed the presence of the organism on at least one of the external surfaces of the teat sphincters immediately before milking, but none were found on the sphincters examined after the cows had been milked and stripped by machine.

Flies were rarely found feeding at the orifice of the teat. It is suggested that likelihood of fly transmission is greater when milk containing the organisms is present on the floor and fundamental sanitation would reduce this possibility. S.A.F.

FEEDS AND FEEDING

752. Feeding of Cows Controls Vitamin A Content of Butter. S. M. HAUGE. Food Indus., *14*, No. 2: 48–49. 1942.

Color is not a true index to the vitamin content of butter. Guernsey and Jersey butters are high in carotene and low in vitamin A; the reverse is true of Ayrshire and Holstein milk and butter.

Winter butters are lower in vitamin A when compared to summer butters. The problem of feeding to maintain vitamin levels in winter butters is difficult. This is due to the lack of hays and silage of excellent quality.

Butter manufacturers will not reduce the vitamin A content of butter when following orthodox procedures. Oxidation reduces the vitamin A contents of butters. J.C.M.

ICE CREAM

753. Ice Cream Vital to Farm Economy. R. C. HIBBEN, Exec. Sec., Internatl. Assoc. Ice Cream Mfrs., Barr Bldg., Washington. Ice Cream Trade Jour., *38*, No. 8: 24. Aug., 1942.

As proof that the ice cream industry is an essential industry from a farm standpoint, the author calls attention to the fact that this year the ice cream manufacturers will use over 6,000,000,000 pounds of milk, two-thirds of which is used during the season of peak production, when the market is needed most for the farmer. In addition to milk 30,000,000 pounds of fruit and 8,000,000 pounds of nuts are used.

Attention is also called to the fact that "Ice cream is a dairy product, a nutritious food; containing 80% by weight of milk and cream and 15%

sweetener, and with added fruit and nuts and other flavor used in ice cream, it is an important food." W.H.M.

754. Corn Syrup Solids: Their Use in Ice Cream. JOHN W. KNECHTGES AND H. H. SOMMER, Dept. Dairy Indus., Univ. Wis., Madison, Ice Cream Trade Jour., *38*, No. 7: 14. July, 1942.

An investigation was made to determine the extent to which corn syrup solids can be used with sucrose in ice cream and the effect of such combinations on the quality of the ice cream.

Corn syrup solids lower the freezing point of mixes less than sucrose. If 4 pounds of sucrose are replaced by 5 pounds of corn syrup solids (1% higher total solids in mix) the freezing points are practically alike. If the total solids content of the mix is kept uniform by cutting the serum solids content, 4 pounds of sucrose may be replaced by 6 pounds corn syrup solids without any practical difference in freezing point.

Corn syrup solids, while acid in reaction, have such a low buffer capacity that they do not affect the acidity of the mix to any significant degree.

The extent to which sucrose may be replaced by corn syrup solids is from 25 to 33⅓% as judged by taste, body, texture and melting behavior of the ice cream.

The whipping ability of mixes is not affected by replacement of sucrose by corn syrup solids.

As judged by melt-down tests, corn syrup solids do not exert a stabilizer sparing action in ice creams with moderate stabilizer content, but in heavily stabilized ice creams the stabilizer content may have to be reduced as much as 25% when 4% of the sucrose is replaced by 5% corn syrup solids.

While slightly favorable effects in delaying sandiness and shrinkage have been observed for corn syrup solids, these effects are too slight to be of practical significance.

In comparisons of 16% sucrose ice cream with 12% sucrose plus 5% corn syrup solids ice cream by consumer preference tests no significant preference was found. W.H.M.

755. Conserving Sugar in Chocolate Ice Cream. B. I. MASUROVSKY, Res. Editor. Ice Cream Trade Jour., *38*, No. 6: 25. June, 1942.

The rationing of cane and beet sugar has caused ice cream manufacturers to use other available sweeteners such as corn sugar, corn syrup solids, honey, malt syrup, maple syrup, sorghum syrup and molasses. Malt syrup which contains 25% moisture, 65% reducing sugars calculated as maltose, 9% dextrine and 1% ash is recommended for use in chocolate ice cream to replace 25% of the sugar of the mix. A good grade of molasses could also be used. In this case it would take 100 pounds of molasses to replace 60 pounds of cane sugar. W.H.M.

756. Wartime Production Problems. P. H. TRACY, Univ. Ill., Urbana. Ice Cream Trade Jour., *38*, No. 8: 12. Aug., 1942.

A number of new problems have been presented to the operators of dairy plants as a result of the war. One of the most serious problems which seems to be common to all plants is the one of labor shortage. Higher wages paid to skilled laborers by defense industries, fewer college graduates and the induction of men into military service have made serious inroads into the labor supply. Milk productions have been increased in response to the demand for evaporated milk, cheese and milk powder, and in many instances this increased flow of milk has resulted in plants being operated at peak capacity which puts an additional strain on plant equipment. Due to a shortage of farm labor it has been difficult to maintain quality of the new material.

The rationing of scarce materials and supplies has forced the ice cream industry to operate on a reduced volume of sugar and chocolate and delivery services have been curtailed to meet the demands created by the rubber shortage. Market milk plants likewise have had to make many adjustments in their delivery services to meet the rubber and labor shortage.

Some operators even with an increased volume of business, are finding it difficult, in face of higher wages, increased taxes and ceiling prices on some products, to operate their plants profitably. W.H.M.

757. Chemical Sterilization of Equipment. B. I. MASLOWSKY. Ice Cream Trade Jour., *38*, No. 9: 29. Sept., 1942.

The rationing of fuel oil will necessitate some ice cream plants to reduce the amount of steam used. Since a large percentage of steam in an ice cream plant is used for sterilizing purposes, the use of chemical sterilizers offers a means of reducing the steam and therefore the oil requirements.

Chlorine sterilizers have been in common use in creameries for many years and their use is safe, convenient, and effective. The shortage of fuel further promotes their use.

In using chlorine sterilizers, the fundamental basis of success is cleanliness before the sterilizing agent is applied, since organic materials, present as grease and dirt, readily inactivate the chlorine. The increasing use of "wetting agents" in cleansing compounds and sterilizing agents helps prevent waste of chlorine. One hundred parts per million of active chlorine, by weight, are sufficient in a solution used to sterilize freezer, pipe lines, vats and average ice cream plant equipment. A new chloride disinfectant is on the market and it may prove to be another step forward in chemical sterilization. This product known as Roccol, alkyl-dimethyl-benzyl-ammonium-chloride, (1–5000 or 200 p.p.m.) is suggested for use in the sterilization of wiping cloths, to avoid transmission of micro-organisms to frozen products. W.H.M.

758. Experiments in Freezing Cream for Storage. G. M. TROUT, Mich. Agr. Expt. Sta., East Lansing, Mich. Canad. Dairy and Ice Cream Jour., *21*, No. 7: 22. 1942.

The purpose of freezing cream is largely to utilize without churning surplus cream at peak of production for later use when fat production is not so great, to conserve fat in plants which do not have facilities for churning, and to provide a more economical fat supply inasmuch as a lower price fat is being stored and that fat losses are reduced to a minimum since churning losses are not involved. The two major problems of freezing cream are the preservation of good flavor and the prevention of de-emulsification of the fat. Cream intended for freezing should have a high fat content, an excellent flavor as the cream after storage will never have a flavor superior to that when fresh, and a low titratable acidity with no developed acidity. It should preferably be high in carotene because such cream is high in color and rich in vitamin A. Results of studies indicate that cream to be stored frozen must be pasteurized at an exposure of not less than 165° F. for 15 minutes and preferably at 185° F. for five minutes. Homogenization had very little inhibitory action toward oxidation. The type of container in which cream may be stored is of little consequence so far as off flavor development was concerned. No method was found whereby the fat emulsion remained completely stabilized upon freezing. Only high-fat, excellent quality, low-acid cream, free from copper contamination should be considered for freezing. Pasteurizing such cream at a high temperature is to be preferred over low-temperature pasteurization. O.F.G.

759. The Proper Care and Use of Homogenizers in Making Ice Cream Mix. A. W. FARRALL, The Creamery Package Mfg. Co., Chicago. Canad. Dairy and Ice Cream Jour., *21*, No. 6: 26. 1942.

Homogenization breaks up fat globules into smaller sizes and has an effect on protein stability and water binding power of the ice cream mix. Recent studies show that greater protein stability is obtained when the fat breakup is secured without excessive pressures. The proper homogenizing pressure to use will depend upon the composition of the mix, the quality of the raw materials, the type of homogenizer, and other factors. The homozenizer should give a uniform steady pressure, which means that the suction and discharge valves must be kept in good condition. There must be no air leaks. Plunger packing should be removed daily for cleaning and when put in place again should be tightened only sufficiently to prevent leaks. The homogenizing valve requires periodic resurfacing. The drive end of the homogenizer usually requires only periodic change of lubricating oil. The homogenizer should be completely dismantled and washed each day in use. Care of the homogenizer is for the purpose of keeping the machine clean and maintaining it in efficient working condition. O.F.G.

760. Reducing Oiling off of Frozen Cream Used in Ice Cream. J. C. HENNING, Div. Dairying, N. Y. Agr. Expt. Sta., Geneva. Ice Cream Trade Jour., *38*, No. 7: 20. July, 1942.

It has been shown that homogenization alone and homogenization and the addition of gelatin did not prevent destabilization and subsequent oiling off by increasing the viscosity and clumping of the fat globules of the cream before storage. The cream separated from pasteurized milk which had been cooled to 40° F., held at that temperature for 20–24 hours, warmed to 80°–85° F. and separated did not oil off as much as cream separated from pasteurized milk which had been cooled to 80°–90° F. The cream from the treated milk oiled off less than the regular cream which had 10% sugar added. It was concluded that differences in the physical state of the cream other than the increase in viscosity was responsible for differences in oiling off after freezing. W.H.M.

MILK

761. Sanitation of Milk Plant Operations Pays Dividends. JAMES A. TOBEY, Amer. Inst. Baking, N. Y. Jour. Milk Technol., *5*, No. 4: 215. July-Aug., 1942.

The cost of sanitation is only a very small fraction of the cost of production and distribution. On the farm the principal cost of milk production is for feed and labor, which are nearly two-thirds of the total cost. The distributors' largest items of expense are his labor and payment to the farmer for the milk. These items represent about 70% of the dealers' total cost.

In Michigan a study made on 499 farms during 1932–1936 reported that the cost of producing milk per cow per year for market milk purposes was $1.22 more than for condensery milk.

In a more recent study made by a large dairy concern it was reported that on 376 farms in eleven markets it cost slightly more than 33 cents a hundred pounds to produce market milk complying with the milk ordinances in force as compared with the cost of producing manufactured milk; in this case evaporated. About 24¢ of this cost was for ordinance compliance and nine cents for extra transportation. Everlasting vigilance in the hygienic production of milk is necessary; many have been the penalties of poor sanitation. A number of examples are cited where courts have awarded damages to consumers due to illness caused by unwholesome or contaminated milk. L.H.B.

762. The Administrative Public Health Problems in Milk and Milk Products. LLOYD ARNOLD, Dept. Bact. and Pub. Health, Col. Med., Univ. Ill., Chicago, Ill. Jour. Milk Technol., *5*, No. 4: 229. July–Aug., 1942.

"Healthy cows, cleanliness and proper cooling are the underlying factors

in farm sanitation. Every administrative office should separate the commercial advantages of increasing keeping qualities versus the protection of the health of the public."

Improvement in rural sanitation has been so marked during the past two decades that it is now possible to shift our administrative control from the farm to the plants. "The facilities for proper milk production may be ascertained by farm inspection which can be made less frequently than when we attempted to control the sanitary quality of the milk on the farm."

Present regulations regarding pasteurization should be continued. More emphasis should be placed on post pasteurization handling of the milk, and better packaging is desirable. "The less inspection and regulation we can do to insure a wholesome, sanitary and drinkable milk, the better off we are in the long run." L.H.B.

763. The Freezing Point of Milk. I. The Freezing Point and Solids-Not-Fat Content of the Milk of Individual Cows Throughout a Period of Lactation. R. ASCHAFFENBURG AND P. L. TEMPLE. Natl. Inst. Res. in Dairying, Univ. Reading, Eng. Jour. Dairy Res., *12*, 315–321. 1941.

The freezing point and solids-not-fat content of individual samples of morning and evening milk from three Dairy Shorthorn cows were determined throughout the greater part of a lactation (March-Oct., 1936). The average values of the freezing points for the three cows were – 0.546, – 0.546 and – 0.544° C. (31.0172, 31.0172 and 31.0208° F.) for the morning milk and 0.548, – 0.548 and – 0.546° C. (31.0136, 31.0136 and 31.0172° F.) for the evening milk. The corresponding averages for the solids-not-fat were 8.84, 8.64 and 8.19% for the morning milk and 8.91, 8.04 and 8.14% for the evening milk.

For each animal the freezing point of the morning milk was on the average 0.002° C. (0.0036° F.) higher than that of the evening milk.

No freezing point higher than – 0.529° C. (31.0478° F.) or lower than – 0.560 C. (30.9920° F.) was encountered. The freezing point in general deviated no more than 2% and in the most extreme case, by no more than 3.5% from the mean of – 0.546 ± 0.002° C. (31.0172° F.). No evidence was found of any influence of the stage of lactation on the freezing point, but a slight increase persisting for some weeks occurred coincident with the time at which spring pasture became available to the animals. S.T.C.

764. The Plate Count and Methylene-Blue Reduction Test Applied to Milk. H. BARKWORTH, South Eastern Agr. Col., Whe, Kent, J. O. IRVING, London School of Hygiene and Trop. Med., AND A. T. R. MATTICK, Natl. Inst. Res. in Dairying, Univ. Reading, Eng. Jour. Dairy Res., *12*, 265–314. 1941.

The primary object of this experiment was to find whether the standards

set by the modified methylene-blue reduction test (5½ hr. winter and 4½ hr. summer) approximated to those set by the plate count method (200,000 colonies per ml.) previously used. A comparison of the grading by the two methods is given as follows:

	Summer samples		*Winter samples*	
	Number	%	*Number*	%
Passed or failed both tests	1003	87.3	631	84.6
Passed plate count, failed R. T.	105	9.1	87	11.7
Passed R. T., failed plate count	41	3.6	28	3.8

A number of samples representing subsamples from a common bulk were tested in their different laboratories. The conclusion is reached that when transit hazards are eliminated, and when standard technique is adhered to rigorously the results of the plate count, the methylene-blue test and the fat test (Gerber) are comparable between different laboratories.

Samples of morning milk were tested in quintuplicate by both plate count and methylene-blue test. The methylene-blue tubes were observed every 5 minutes. One hundeed sixty-nine samples were so analyzed. Defining sensitivity as the power of discriminating significantly between milks of different bacterial constitution, the conclusion was reached that the plate count is slightly more sensitive than the methylene-blue test observed every 5 minutes and slightly more sensitive than the latter observed every half hour. Since the coefficient of variation of the logarithm of the plate count was slightly less than that of the methylene-blue test observed every 5 minutes it is considered to be the correct measure to use. A further conclusion was that two samples whose reduction times differ by 30 minutes are not significantly different. Such a difference is only significant if the result is based on the mean of duplicate tests with 5-minute observations or of triplicate tests with half-hourly observation. S.T.C.

765. The Stability of Homogenized Milk. V. B. Sullam, N. C. State Col. Milk Plant Monthly, *31*, No. 4: 23. April, 1942.

Samples of milk testing 3.5% and 4.6% were taken (1) immediately after standardization, (2) after pasteurization at 160° F., and (3) after similar pasteurization but homogenized at 2500 pounds pressure and studied for protein stability, using protein precipitants such as alcohol, acetone, several neutral salts, aluminum sulphate, aluminum chloride, lactic and hydrochloric acid. The results obtained with alcohol, acetone, and neutral salts indicated that homogenization increased the protein stability of milk whereas, results from the other chemicals used indicated no difference in stability between raw, pasteurized and homogenized milk.

Homogenized milk could be identified by adding to a 5-ml. sample of the milk under question 2 ml. of a saturated solution of common salt and 8 ml. of distilled water. A positive test shows no separation.

The conclusion is reached that no special difficulty in the manufacturing, handling, and trading of homogenized milk be expected as a result of effects upon stability. G.M.T.

766. Elasticity of Supply of Milk from Vermont Plants. III. Forecasting the Milk Supply. S. M. JOHNSON, Vt. Agr. Expt. Sta. Bul. 480. 1942.

The purpose of this study was to determine the manner in which the supply of milk in Vermont has responded to changes in various factors, and to arrive at a more satisfactory method of forecasting the milk supply. Data on (1) how changes in production are brought about, and (2) on which they are brought about may be used as a basis of prediction.

Of the first type, most valuable for purposes of prediction are data on changes in cattle numbers. The number of milch cows can be predicted with considerable accuracy a year in advance, from data on the number of one- to two-year-old heifers on hand, and on recent slaughter and interstate shipments of cows. The rate at which grain is being fed is highly correlated with current, but not with future, production, and is of little or no value as a basis for prediction of receipts.

Of the second type of data, of things which influence farmers to change the numbers of cows milked or feeding practices, and so to change the amount of milk produced and delivered to plants, the relation between milk prices and grain prices, the milk-feed price ratio, has proven to be most closely related to subsequent production, and most useful for purposes of prediction of supply.

Of the various forms and combinations of factors examined in this attempt to develop a technique for accurate forecasting of the total market supply of milk in a producing area, the group which gave the best results (Method 11, p. 58) included factors of both types. P.H.T.

767. Effect of Homogenization on the Curd Tension, Digestibility, and Keeping Quality of Milk. C. J. BABCOCK. U.S.D.A. Tech. Bul. 832. 1942.

A pressure of 2500 gives the maximum reduction in curd tension obtainable. Milk homogenized after pasteurization will have a lower curd tension if processed at a temperature either higher or lower than the temperature of pasteurization. There is no advantage of the two-stage homogenizer over the single-stage machine as far as curd-tension reduction is concerned. An increase in butterfat results in a reduced curd tension. A mixture of homogenized and unhomogenized milk produces a product with a curd tension slightly lower than the theoretical average curd tension for that particular combination. Partial freezing does not materially effect the curd tension of milk.

By digestion in vitro, it was found that proteolysis takes place more rapidly in either boiled or homogenized milk than in raw milk but not more completely.

In the presence of artificial gastric juice homogenized milk forms smaller curds than unhomogenized milk.

Homogenization retards or inhibits the development of copper induced oxidized flavor in milk. Approximately 10 times as much copper is required to produce an oxidized flavor in homogenized milk as is required to produce a similar flavor in the same milk unhomogenized.

Homogenized milk is more susceptible to the action of sunlight than is unhomogenized milk.

From a bacteriological standpoint (as determined by the plate count) there is no significant difference in homogenized and unhomogenized milk. The extent of acid development in those two types of milks is similar

P.H.T.

768. The Bacterial Population of Paper Milk Containers in Relation to Methods of Moisture Proofing. J. W. RICE, N. Y. Agr. Expt. Sta., Geneva. Tech. Bul. 263. 1942.

Thirty-two hundred Pure Pak milk containers were made up and sealed under commercial conditions for experimental purposes in 16 series of 200 containers each. Each series of containers received special moisture-proofing in the form of a specific melting-point paraffin applied at a given temperature. In the laboratory, under random sampling, 100 containers from each waxing treatment were subjected to nutrient broth sterility tests, and an additional 276 containers were tested by plating sterile water rinses to obtain bacterial counts. In both of these methods the sterile charges of water and of broth were introduced by a special syringe-needle-puncture method to avoid possible air contamination.

The efficiency of wax coverage obtained in the several moisture-proofing processes was studied by making microscopic examinations and counts of punctiform spots on the inside walls and by planimetric measurements of stained corners and seams on the insides of containers which were treated by 24-hour contact with a 0.1% methylene-blue solution at room temperature.

From the broth sterility tests made on a total of 1600 containers, selected uniformly from the several moisture-proofing treatments, it was found that approximately 80% of them were sterile. The temperature at which moisture-proofing was done, as well as the melting-point of the paraffin used in the various treatments, showed practically no differences in the percentage of sterility among the several sets of containers. Plate counts of viable bacteria from the platings of water rinses made on 276 containers, chosen from the moisture-proofing processes, revealed approximately 60% sterility.

Only 10 containers yielded more than five colonies per container. The moisture-proofing methods employed showed no effect on the percentage sterility nor on average colony counts per container by this method of testing.

Planimetric readings of methylene-blue-stained corners and seams and counts of punctiform spots on the inside walls of 320 containers selected uniformly from all methods of moisture-proofing indicate quite clearly that the most efficient moisture-proofing is obtained by treating paper containers with a high-melting-point wax, such as 133° to 135° F. American melting point applied at the lowest possible temperature, such as 150° F. P.H.T.

769. **How Methods and Promptness of Cooling Affects the Quality of Milk.** A. J. Gelpi, C. S. McCleskey, D. M. Smith, La. State Univ. Univ. Bul. 344. 1942.

The following methods of milk cooling were studied: (1) Cooling tank using 33°–40° F. water agitated. (2) Conical cooler to 33°–36° F. followed by storage in can in water tank (33°–40° F.). (3) Tubular surface cooler to 33°–36° F. followed by storage in can in water tank at 33°–40° F.

It was found that the milk cooled in the can in the tank (method 1) had the highest bacterial content. Delayed (2 hours) cooling was of no significance in the case of methods 2 and 3 but resulted in a significant increase in bacteria in the milk cooled by method 1 after holding 12 hours. There was no difference in the flavor of the milks that could be attributed to the method of cooling.

Directions for the production of high-quality milk are included.

P.H.T.

770. **Bacteriological Control of Milk Quality.** Lawrence Little, Sterling Meadow Gold Dairy, Oklahoma City. Jour. Milk Technol., *5*, No. 4: 221. July–Aug., 1942.

Tests were made on samples of both inspected and uninspected milks over a period of 14 months for determining the presence of thermoduric organisms.

It was found that thermoduric organisms were prevalent in both supplies. In the uninspected milks they were present the year round, while in the inspected or graded milks they were of little consequence in the summer but were a problem in the winter.

Microscopic examination of incubated samples of raw milk was of no value in detecting the presence of thermoduric organisms. Neither was the fermentation test of any value. The methylene-blue and resazurin tests were also found to be ineffective for detecting samples with large numbers of thermoduric organisms.

Laboratory pasteurization is necessary for detecting these organisms. It definitely detects a type of unsanitary conditions on the farm that is not

detected by any of our present tests. However, it in itself is not a sufficient test for determining the sanitary quality of a milk supply. Meyers and Pence's oval tube technique was used in connection with the pasteurization test and proved its worth. It was found that it could replace the standard agar plate count altogether for routine control work, saving labor, equipment and space. L.H.B.

771. **The "Cream Top" Type Bottle for Laboratory Sampling of Homogenized Milk.** JOSEPH LEVINE AND RUBIN H. FEINGOLD, Bur. Food and Drugs, Dept. Health, N. Y. Jour. Milk Technol., *5*, No. 4: 202. July–Aug., 1942.

A simple and practical method for sampling homogenized milk for determining the fat content in the upper 100-ml. layer is described and illustrated.

Homogenized milk, when brought into the laboratory, is thoroughly mixed and then poured into the cream top bottle; placed in the refrigerator and allowed to set undisturbed for 48 hours, at end of which time a plunger-type apparatus which is illustrated in the article is inserted in the bottle so that it forms an effective seal in the neck, permitting the pouring off of the upper layer. A volume of approximately 100 ml. is obtained in this portion. L.H.B.

772. **The Influence of Ammonia on the Development of Rancidity in Milk.** C. H. CASTELL, Dept. Bact., Ontario Agr. Col., Guelph, Ontario. Jour. Milk Technol., *5*, No. 4: 195. July–Aug., 1942.

Preliminary experiments conducted showed that both addition of ammonia to milk and the exposure to an atmosphere containing ammonia were effective in accelerating the reduction in surface tension and in the development of rancid flavors when milk was held at 5° C.

It was found that there was an apparent difference in effect on the milk from different cows, and to a lesser degree with different breeds. The effect increased as the lactation period advanced.

The author doubts if the concentration of ammonia, except in extreme cases, in the atmosphere of the barn where cows are milked would ever be a major factor in causing the development of rancidity in milk and cream. However, in late winter and early spring a strong odor of ammonia is not uncommon in many barns, and could very well be a contributing factor. Also, a breakdown of a refrigeration unit, accompanied by an escape of gas in the atmosphere, could be a factor in accelerating the development of rancidity in raw milk and cream. L.H.B.

773. **Thermoduric Organisms in Relation to High-Temperature Short-Time Pasteurization.** F. W. FABIAN, Res. Prof. Bact., Dept. Bact. and Hygiene, Mich. State Col., East Lansing. Jour. Milk Technol., *5*, No. 4: 237. July–Aug., 1942.

"Thermoduric in dairy bacteriology is used to designate a group of

bacteria which will withstand the temperature of milk pasteurization, 140 to 145° F. for 30 minutes, or 160–161° F. for 15 to 16 seconds, but will not grow at this temperature."

We should expect to find more thermophilic bacteria in milk pasteurized at 142–145° F. for 30 minutes than in milk pasteurized at 160–161° F. for 15–16 seconds, and conversely we should expect to find more thermoduric bacteria in milk pasteurized at 160–161° F. for 15–16 seconds than in milk pasteurized at 142–145° F. for 30 minutes.

Four groups of these latter mentioned organisms are commonly found in milk, being micrococci, streptococci, sarcinae and bacilli. Most common are the micrococci.

The principal sources of these organisms are the udder, poorly cleaned and improperly sterilized milking machines, milk pails, cans and other milk utensils.

Methods most generally used for determining the presence of thermoduric bacteria are laboratory pasteurization of the milk samples, then plating, or laboratory pasteurization at 161° F. for 16 seconds, then incubating at 37° C. for seven hours, after which they are examined under the microscope. Another method is to incubate the raw milk at 58° to 60° F. for two hours and then examine under the microscope.

Thermoduric bacterial control is a producers' problem. L.H.B.

774. **Problems Incident to the Production and Use of Homogenized Milk.** G. M. TROUT, Mich. Agr. Expt. Sta., East Lansing, Mich. Jour. Milk Technol., *5*, No. 4: 233. July–Aug., 1942.

The problems involved are processing, packaging, distribution, laboratory control, cooking and utilization of returns.

Processing creates problems which require attention, such as processing temperature, clarification, bacterial counts, cream line and efficiency of homogenization treatment in order to meet required standards.

Packaging problems are created due to the fact that homogenization increases foaming, making it difficult to fill the packages properly.

Distribution problems encountered are seepage around cap seat in the summer, and in winter, watery appearance of the upper portion when thawed after freezing.

Laboratory control: Modification of the Babcock test is required. H_2SO_4 acid, having a sp. gr. of 1.815–1.820, is recommended.

Efficiency of homogenization must be checked.

Cooking: Problems arising in the use of homogenized milk in cooking deal chiefly with the fact that it is apparently more sensitive to heat than unhomogenized milk.

Utilization of returns: This is not so much of a problem as when homogenization was first introduced. It has been shown that homogenized milk can be successfully used in making cottage cheese and buttermilk. L.H.B.

775. Merits of Churned Buttermilk. C. L. ROADHOUSE, Univ. Calif., Davis, Calif. Canad. Dairy and Ice Cream Jour., *21*, No. 8: 54. 1942.

The following points are suggested for making a churned buttermilk that is pleasing in flavor, does not "whey off" readily, contains butter granules, and between 1 and 2% fat: (1) Use a clean-flavored vigorous starter, (2) Select skim milk of the best quality, (3) Pasteurize so as to destroy as many bacteria as possible, (4) Pasteurize the cream separately at 145° F. for 30 minutes, (5) Mix the cream with the skim milk before or after the starter is added, (6) Add 0.75% fat in the form of cream, (7) Develop an acidity of 0 7 to 0.8%, (8) Add a small amount of salt to improve the flavor, (9) Churn at a temperature between 68 and 72° F., and (10) add to the churned buttermilk at least 0.025% sweet cream. O.F.G.

776. Adjusting Milk Delivery to Meet Tire Shortage. W. P. MORTENSON, Univ. Wis., Madison, Wis. Canad. Dairy and Ice Cream Jour., *21*, No. 8: 29. 1942.

This is a timely article dealing with ways and means of conserving tires in the dairy industry. The author suggests that the following services can be discontinued: (1) Collecting milk accounts and soliciting new customers, (2) Special delivery, and (3) Special brands of milk and specialty products. Other services can be materially reduced and the author suggests the following five plans: (1) Zone the city for home milk delivery, (2) Form an independent unified delivery system for the entire market, (3) Every other day milk delivery, (4) Eliminate home delivery entirely and let consumers obtain milk from retail stores and milk depots, (5) Unify the whole milk distribution system, that is (a) set it up as some kind of public utility like gas, street car, bus, electric system, or (b) let the city own the milk distribution as it now does the water system.

None of these plans will bring about a complete solution satisfactory to all interests in the market. O.F.G.

777. Canadian Agriculture in the Post-War Period. J. F. BOOTH, Assoc. Dir. of Marketing, Dept. Agr., Ottawa, Canada. Canad. Dairy and Ice Cream Jour., *21*, No. 5: 30. 1942.

A 200-year chart of price levels shows a gradual improvement of farm prices relative to general wholesale prices. In each war period, prices increased more than 100% and in each case they returned to a level close to, or below, that prevailing before the war. There is certain evidence that agriculture has been adversely affected by the outcome of wars. An increase or decrease in retail prices of 20% may mean a 30% change in wholesale prices and a 50% change in farm prices. Such a relationship indicates

that farmers are much more drastically affected by price changes than most other groups. This characteristic of farm prices is closely related to a failure of farmers to quickly adjust production levels after a period of inflation and high production level. The author's interpretation of past records brings the conclusion that wartime inflationary prices have been distinctly harmful to agriculture. Farmers have every reason to join with others in a program of price stabilization. The post-war period will be influenced by demobilization and soldier settlements, adjustments in industry and labor, international trade relations, immigration policies, and reconstruction abroad. Farmers should not oppose a policy that involves the admission of immigrants representing various occupations in somewhat the same proportion as such occupations are now represented in the Canadian population. The question of markets in the post-war period is of great importance to Canadian agriculture and most economists favor the removal of trade barriers in order to increase these markets. The opportunities of expanding the domestic Canadian market for certain foods is good since a substantial proportion of Canadian people do not obtain a sufficient supply of certain foods. O.F.G.

778. A New Order in the Making for Canadian Dairymen. J. E. LATTIMER. Canad. Dairy and Ice Cream Jour., *21*, No. 4: 25. 1942.

This is an address given before the National Dairy Council of Canada. The author points out that Canada ranks high in per capita consumption of dairy products because of its large consumption of butter. Canadians in general pay less for their fluid milk than do United States consumers. The provision of cheap milk by the producer is primarily due to the great number of milk cows in proportion to population in Canada, the small dependence on hired labor, and the ability and persistence of the dairy farmer. Price regulation of fluid milk has come into existence primarily to insure a regular supply to the consumer. Regulation has been extended recently to cheese and butter. If a new order for the farmer in general and the dairy farmer in particular is wanted, the first need is a new deal in the matter of wages. Past records indicate that a new order is necessary. The following points must be considered in establishing a new order for the dairy farmer: 1. Decentralization of butter making, 2. Reduced costs of distribution of fluid milk, 3. Raising dairy heifers in less expensive surroundings, 4. Artificial insemination, 5. Improved pastures, 6. Increased volume of production per farm, 7. Cheaper feeds. O.F.G.

PHYSIOLOGY

779. Studies on Experimental Teat and Mammary Development and Lactation in the Goat. S. J. FOLLEY, HELEN M. SCOTT WASTON,

AND A. C. BOTTOMLEY. Natl. Inst. Res. in Dairying, Univ. Reading, England. Jour. Dairy Res., *12*, 241–264. 1941.

The authors summarize their results as follows:

1. Observations on five animals indicate that the teats of immature castrated male goats grow isometrically. Less extensive data on three animals allow of the tentative conclusion that the same is true of immature normal males. In the male goat, therefore, teat growth appears to be uninfluenced by the testes.

2. Administration of diethylstilbestrol or its dipropionate to normal or castrated immature male goats causes the teats to grow allometrically for a time.

3. No externally visible udder growth occurred even when oestrogen treatment was prolonged for periods of over a year and was supplemented by progesterone or ethinyltestosterone. Whole mounts of glands from treated animals indicated that some mammary growth had occurred. In two cases microscopic examination showed the presence of alveoli. No explanation can be offered of the failure to develop the udder in the male goat experimentally.

4. Endocrine activity of the ovary as evidenced by a change from isometric to allometric teat growth often manifests itself in the young female goat at an early age. In one case allometric teat growth was in progress at 41 days of age. During the allometric phase the data agree with the simple allometric law.

5. During the rutting season following its birth, teat growth ceases completely in the female goat; allometric growth is resumed when the rutting season ends. It therefore appears that the corpus luteum inhibits teat growth.

6. Administration of diethylstilbesterol or its dipropionate (by inunction of the udder region) causes, in the virgin female, an increase in the rate of teat growth accompanied by udder growth.

7. In virgin females the above treatment was followed, after a latent period during which udder growth occurred, by prolonged lactation, at the height of which the daily milk yield was of the order of 1·5–2·1. No treatment with anterior pituitary extract was necessary. In normal or castrated males the treatment resulted in the secretion of only minute quantities of milk.

8. Lactation was initiated and increased in intensity while oestrogen administration continued. It is thought that low doses of oestrogen exert a galactopoietic effect through the anterior pituitary.

9. During the first part of the artificial lactation period, the changes in milk composition were similar to those characteristic of the colostral period. Subsequently, milk of excellent chemical quality was secreted. S.T.C.

780. Effects of Testosterone Propionate and Stilbestrol on the Mammary Gland Postpartum. EDWARD M. JEPPSON, HARRY Y. KASABACH AND AARON E. KANTER, Salt Lake City and Chicago. Jour. Clin. Endocrinol. *2*, No. 1: 16. Jan., 1942.

A study was made of 124 patients to determine the value of testosterone propionate in the prevention of pain of breast engorgement in the puerperium. The results showed that testosterone propionate was of little or no value for this purpose and of some disadvantage since it caused delay and discontinuance of lactation. Testosterone and diethylstilbestrol were useful in preventing or discontinuing lactation. R.P.R.

781. Use of Ethinyl Estradiol to Prevent Lactation in Puerperal Patients. LAWRENCE KURZROK, CHARLES H. BIRNBERG, AND SEYMOUR LIVINGSTON, Jewish Hospital and Greenpoint Hospital, Brooklyn, N. Y. Jour. Clin. Endocrinol., *2*, No. 7: 471. July, 1942.

Fifty-nine puerperal women were given ethinyl estradiol orally for 3 to 4 days. When amounts up to 1.5 mg. were given daily the results were only fair, with 9 failures in a series of 26 cases. Excellent results were obtained in 12 cases when 1.5 to 2.4 mg. of ethinyl estradiol were given daily. There was one failure. The administration of 1.8 to 2.4 mg. daily for 3 to 4 days, 4 to 21 days post-partum, inhibited lactation even though the women had begun to nurse their babies. R.P.R.

782. Comparative Clinical Effects of Orally Administered Alpha-Estradiol and Diethylstilbestrol on Post-partum Engorgement of the Breast. A. W. DIDDLE, S. F. NAGYFY, AND R. L. SELLS, State Univ. of Iowa. Jour. Clin. Endocrinol., *2*, No. 5: 307. May, 1942.

Sixty-one post-partum women were given alpha-estradiol in propylene glycol sublingually to test its effect on breast engorgement as compared with that produced by diethylstilbestrol. Comparable dosages of both drugs given over the same period produced similar results provided the alpha-estradiol was given in divided doses at regular intervals throughout the day and night. Initiation of lactation was usually delayed but not inhibited entirely. R.P.R.

783. Selection of Nipples by Suckling Rats and Its Effect upon Mammary System. CHARLES K. WEICHERT, Univ. Cincinnati. Endocrinol., *31*, No. 3: 349. Sept., 1942.

Observations on 67 lactating rats showed that when small litters were being suckled not all teats were used by the young. In only one of over 150 animals suckling 7 or more young was there neglect of any teats. There was a correlation between litter size and the specific teats which were

utilized. Selection of teats took place in an anterior-posterior sequence, litters of one confining themselves to the anterior pectoral region. The mammary parenchyma of neglected teats retrogressed while that associated with suckled teats was fully functional. Prolactin injections failed to maintain complete activity in neglected mammary parenchyma. The importance of careful observation of teats prior to removal of mammary tissue was emphasized. R.P.R.

784. Influence of Thyroxine upon Mammary Lobule-Alveolar Growth. JOHN P. MIXNER AND C. W. TURNER, Univ. Mo. Endocrinol., *31*, No. 3: 345. Sept., 1942.

An optimal dose of thyroxine significantly increased the percentage of ovariectomized mice which responded with mammary lobule-alveolar growth to minimal doses of progesterone plus estrone. The thyroxine administration increased the efficiency of progesterone plus estrone in stimulating mammary lobule-alveolar growth by about 25%. Thyroidectomy inhibited the ability of ovariectomized mice to respond to progesterone plus estrone with mammary lobule-alveolar growth. R.P.R.

785. Studies Concerning Mechanism Controlling Initiation of Lactation at Parturition. IV. Influence of Suckling on Lactogen Content of Pituitary of Postpartum Rabbits. JOSEPH MEITES AND C. W. TURNER, Univ. Mo. Endocrinol., *31*, No. 3: 340. Sept., 1942.

The effect of the nursing stimulus after parturition on pituitary lactogen content was determined in 72 New Zealand White rabbits either by permitting the litters to remain with their mothers or by removing them at birth. On the second day after parturition the pituitary lactogen content of nursed and non-nursed does was approximately the same. There was an abrupt increase in pituitary lactogen content to a peak level on the fifth day postpartum in both groups, however, the pituitaries of the nursed group contained 66% more lactogen than did the pituitaries from the non-nursed group. In both groups pituitary lactogen content had decreased at 10 days post-partum and this was followed by a further decrease at 20 days postpartum. Mammary secretion appeared to be fully established 5 days after parturition although considerably more milk was present in the glands of the nursed rabbits than was present in the glands of the non-nursed rabbits. R.P.R.

786. Lactogenic Hormone Content of Anterior Pituitary Gland of Albino Mouse as Compared to Other Species. VICTOR HURST AND C. W. TURNER, Univ. Mo. Endocrinol., *31*, No. 3: 334. Sept., 1942.

The lactogen content of the pituitary of the virgin female was relatively

low. By the 10th day of pregnancy there was an 88% increase in pituitary lactogen content on the basis of 100 gm. of body weight. This was followed by an additional increase of 63% following parturition.

On the 5th day post-partum pituitary lactogen content was 201% above the virgin female level after which it declined rapidly. At 21 days post-partum the lactogen level was 38% above that of the virgin female. Neither ovariectomy nor thyroxin injections altered the pituitary lactogen content.

R.P.R.

787. Influence of Antagonism Phenomenon on Mammary Gland Development. FRITZ BISCHOFF AND LOUISE P. INGRAHAM, Santa Barbara Cottage Hospital Res. Inst. Endocrinol., *31*, No. 3: 326. Sept., 1942.

Injection of sheep pituitary gonadotropin once daily for 10 days into mature virgin female mice under conditions designed to produce rapid, moderately delayed, or delayed absorption produced a degree of ovarian hypertrophy proportionate to the delay in absorption. Rapid absorption of the hormone caused no significant alveolar development but delayed absorption produced marked alveolar development of the mammary gland.

R.P.R.

MISCELLANEOUS

788. Food Plants Prepare for Air Raids. THOMAS ROBERT EDWARDS, JR. Food Indus., *14*, No. 3. 1942.

Practical precautions taken by food plants for protection against air raids were studied. A coffee plant, doing no work at night, had no blackout facilities, but fire drills were practiced. A bakery was found best prepared.

The bakery had sliding panels to keep light in and flying splinters out. They have sand available and also have appointed fire wardens. They have an auxiliary electric plant and a supply of oil in case the gas goes off. The water supply is guarded in a sealed tank. Their employees have been well trained and know exactly what to do.

The dairy used in the survey also has sand available and has an underground tunnel for protection. The shift engineer has charge of throwing off the switches and the water supply is well protected.

Canneries have done little as they usually operate in rural areas. The operators interviewed were studying further plans.

Meat packers have changed their loading hours, blackout windows are installed, and more guards have been added. Their people are also well trained on what they are to do. An adequate signal system has been set up also.

J.C.M.

789. Army Needs 13 New Foods. MAJOR JESSE H. WHITE. Food Indus. *14*, No. 3: 38–39. 1942.

This article lists the following as desirable for the army: (1) pork sausage patties, (2) liver combinations, (3) beef and noodles, (4) pork hash has possibilities, (5) corned pork and chopped ham, (6) concentrated soups or gravies, (7) meat and spaghetti (not yet approved), (8) Hungarian goulash (from Old World recipe), (9) canned mortadella sausage meat, (10) beef and rice (so far rice not desirable), (11) cheese and bacon, cheese and ham, using both smoked and unsmoked cheese, (12) gelatin coatings for hams and cheese, (13) new packing uses: cellophane, pliofilm, Cry-O-Vac. coverings for meat and cheese to prevent loss of moisture.

J.C.M.

790. Improved Firing Cuts Fuel Bill 38%. FRANCIS A. WESTBROOK. Food Indus., *14*, No. 3: 43. 1942.

Taystee Bread Co., Indianapolis, Ind., saves around $1600 a year since they installed a stoker. With this arrangement one boiler does the work that two had done previously. The pneumatic spreader stoker feeds two boilers at the same time and can be switched in a few minutes. Each boiler has a below-grate fan, pinhole grates and a feed tube into the firebox. A valve shuts off the intake of hot gas. Automatic controls are used for steam, air, etc.

The boiler room can be kept clean, as there is no manual handling of the coal. Coal too large is crushed automatically. The coal is burned more completely and ash is easier to remove. A check made in winter showed no "fly ash" on the snow around the building. J.C.M.

791. Protect Your Plants from Bombs and Sabotage. ANONYMOUS. Food Indus., *14*, No. 2: 37–40. 1942.

This article gives details for destruction prevention after bombings and sabotage. Three documents on blackouts, civilian defense and protection of industrial plants available at a low cost from the Supt. of Documents at Washington are recommended. These can be purchased for twenty-five cents each.

A total of 26 steps are listed in keeping plants free from bomb and sabotage damage.

Functions, fire protection, medical and maintenance services are discussed in detail.

This article is too detailed for abstracting in full. Its value lies in a complete understanding of the entire article and a knowledge of the material in the documents listed as being available to all plant operators. J.C.M.

792. Preventing Spoilage by Mold and Bacteria. E. F. GLOBE. Food Indus., *14*, No. 2: 46–48. 1942.

The characteristics of molds are discussed. The four commonly used inhibitors in the bread industry are discussed as a possibility of finding applications in other industries.

The chemistry of inhibitors is important as their use in various ways is limited by their reaction. All inhibitors accepted do not effect flavor or color; two items of primary importance. J.C.M.

793. Relation of Equipment to Operating Economics. G. D. AMERDING, Mojonnier Bros., Chicago, Ill. Canad. Dairy and Ice Cream Jour., *21*, No. 8: 25. 1942.

A recent survey of 30 receiving stations showed the total operational costs divided up as follows: Waste 1%, fuel 5%, supplies 5%, maintenance 7%, administration 10%, power 11%, fixed expenses 24%, labor 37%. The following suggestions are made on how to prolong the life of equipment: (1) Do not try to save on lubricating oil, (2) Keep the equipment clean, (3) Do not crowd the equipment, (4) Do not abuse the equipment. Other points discussed are care of conveyors, care of sanitary fittings, care of rubber hose, checking of the brine system, care of the separator, disposal of acids, being economical with labor because of the shortage, conservation of electricity, full use of water, saving of fuel, care of electric motors, and care of coolers and cabinets. O.F.G.

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JOURNAL
OF
DAIRY SCIENCE

VOLUME XXV

JANUARY, 1942, TO DECEMBER, 1942

1942

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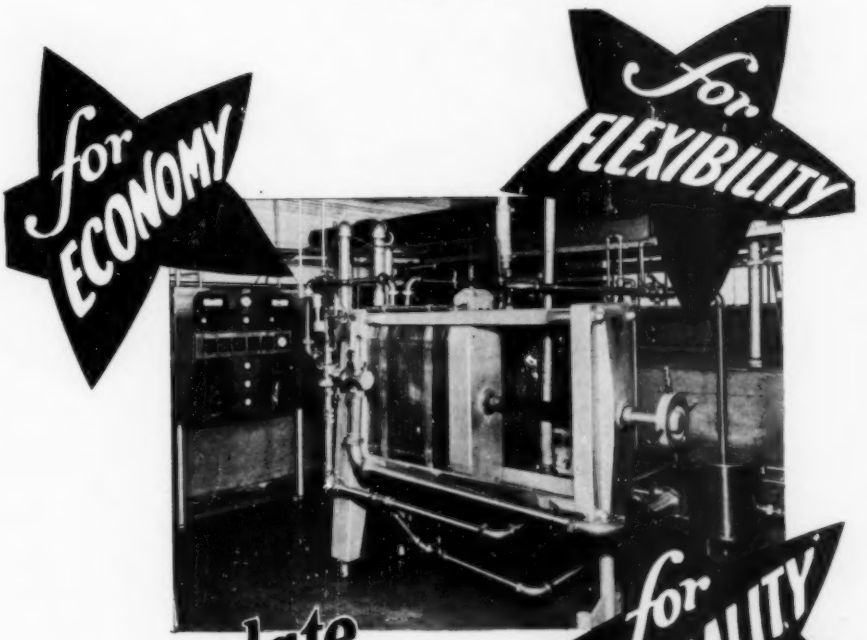

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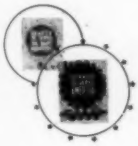

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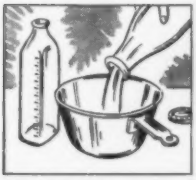

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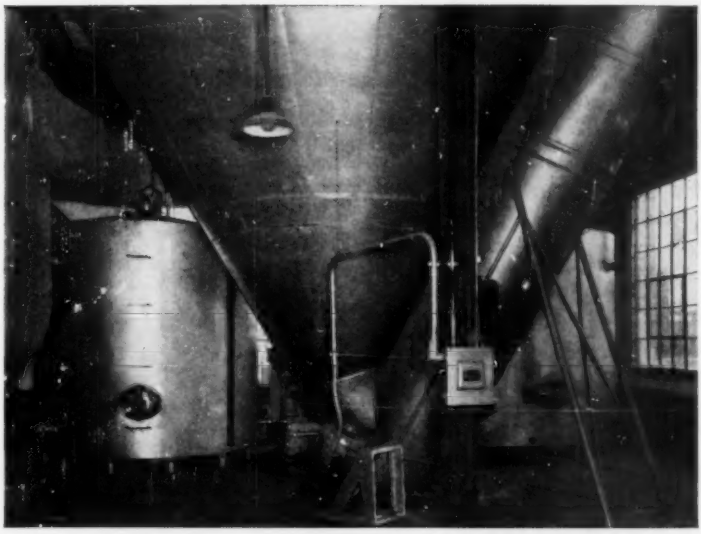

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The American Dairy Science Association was organized to advance the general welfare of the dairy industry, especially by the improvement of dairy instruction by the stimulation of scientific research in all phases of the subject and by improvement in methods of conducting extension work.

Membership. Any person is eligible to membership who is formally announced by an Agricultural College or Experiment Station, or by the Bureau of Dairy Industry of the United States Department of Agriculture or by the Canadian Department of Agriculture as an instructor, extension worker, investigator, or administrative officer connected with the dairy industry, or any person filling a position of responsibility connected with the dairy industry who has had a college or university training in technical science, or any person filling a responsible position in the dairy industry of a professional character requiring a technical knowledge of dairying of a high order. The membership fee is $5.00.

The dues are $5.00 a year, $3.00 of which is for a year's subscription to the *Journal of Dairy Science.* Correspondence regarding membership and dues should be addressed to R. B. Stoltz, Ohio State University, Columbus, Ohio.

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